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Title: Cathodal transcranial direct current stimulation of the extrastriate visual cortex modulates implicit anti-fat bias in male, but not female, participants

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Section/Category: Behavioral and Cognitive Neuroscience

Keywords: anti-fat bias; Extrastriate visual cortex; tDCS; Implicit Association Test

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Abstract: Explicit negative attitudes towards obese individuals are well documented and seem to modulate the activity of perceptual areas, such as the Extrastriate Body Area (EBA) in the lateral occipito-temporal cortex, which is critical for body-shape perception. Nevertheless, it is still unclear whether EBA serves a role in implicit weight-stereotypical bias, thus reflecting stereotypical trait attribution on the basis of perceptual cues. Here, we used an Implicit Association Test (IAT) to investigate whether applying transcranial direct current stimulation (tDCS) over bilateral extrastriate visual cortex reduces pre-existing implicit weight stereotypical associations (i.e. "Bad" with Fat and "Good" with Slim, valence-IAT). Furthermore, an aesthetic-IAT, which focused on body-concepts related to aesthetic dimensions (i.e. "Ugly" and "Beautiful"), was developed as a control condition. Anodal, cathodal, or sham tDCS (2 mA, 10min) over the right and left lateral occipito-temporal (extrastriate visual) cortex was administered to 13 female and 12 male participants, before performing the IATs. Results showed that cathodal stimulation over the left extrastriate visual cortex reduced weight-bias for the general evaluative (Bad vs. Good) but not specific aesthetic (Ugly vs. Beautiful) dimensions as compared to sham stimulation over the same hemisphere. Furthermore, the effect was specific for the polarity and hemisphere of stimulation. Importantly, tDCS affected the responses only in male participants, who presented a reliable weight-bias during sham condition, but not in female participants, who did not show reliable weight-bias at sham condition. The present results suggest that negative attitudes towards obese individuals may reflect neural signals from the extrastriate visual cortex.

Response to Reviewers: Dear Editor,

thanks for considering our paper as acceptable for publication in Neuroscience.

Figures have now amended so to conform to the editorial requirements.

We look forward to hearing from you at your earliest convenience.

Kind regards,

Dr Valentina Cazzato

Neuroscience – Ms. No.: NSC-17-290

Title: Cathodal transcranial direct current stimulation of the extrastriate visual cortex modulates implicit anti-fat bias in male, but not female, participants

Section: Behavioral and Cognitive Neuroscience

Revision Due Date: 05/29/2017

Authors: Cazzato, Valentina; Makris, Stergios; Urgesi, Cosimo

Dear Prof. Juan Lerma and Dr. Santiago Canals,

We thank you and the Reviewers for the positive evaluation and acknowledge the comments helped us to improve the MS very much. In the current revision, we took into account all the points raised by the two Reviewers and made the appropriate changes and adjustments in an integrated way. We are confident that, thanks to the Reviewers' comments, the new version of the manuscript has greatly improved.

Our point-by-point responses to the Reviewers (also uploaded as a supplementary file for review) are marked in bold. Changes to the MS are highlighted in bold to facilitate identification. We do hope that you and the Reviewers will find the revision satisfactory so to finally support our paper for publication in Neuroscience - Section: Behavioral and Cognitive Neuroscience.

We look forward to hearing from you at your earliest convenience.

Kind regards,

Dr Valentina Cazzato

Response to Reviewers - Ms. No.: NSC-17-290

Reviewer #1: The study by Cazzato et al. offers a causative, tDCS-based, approach to the ongoing literature regarding the neural basis of the anti-fat bias. The paper reports findings from a study in which a group of male and female young participants were engaged in a IAT task measuring anti-fat bias and in a (control) aesthetic-IAT following cathodal, anodal, or sham tDCS over the right extrastriate visual cortex in one day, and over the left extrastriate visual cortex in another day. Results showed that cathodal-tDCS over extrastriate visual area in the left hemisphere significantly reduced the anti-fat bias in male (but not in female) participants. The same stimulation did not affect the control aesthetic IAT (in either male or female participants). Anodal stimulation of left or right extrastriate visual cortex and cathodal stimulation of the right extrastriate had no effect in either IAT.

Overall, I think this is an interesting paper that critically adds to prior literature regarding the neural bases of social stereotypes related to body-appearance.

Comment No. 1: We thank the reviewer for his/her positive evaluation of our paper and for his/her insightful queries and helpful suggestions that we have been happy to use in order to improve the MS.

I just have a few minor issues that the authors may want to consider:

- Do the authors think that the different output of cathodal/anodal stimulation may have depended on order of tDCS sessions (anodal first or cathodal first)? May have order of IAT (anti-fat bias or aesthetic IAT first) also affected somehow the results?

Comment No. 2: This is a very interesting point and we are grateful to the Reviewer for raising it. As described at pages 13 and 14, we have reported that the order of the hemisphere daily sessions and of the tDCS stimulation-condition blocks were counterbalanced across subjects. Furthermore, an interval of 3-5 days between the two daily sessions and of at least 90 min was allowed between sessions. This was aimed at ruling out that expectedly likely learning and repetition effects could affect the obtained results. Indeed, in agreement with Mancini et al., 2012; Bolognini, et al., 2010; 2011' studies, the order of stimulation polarity was randomized and counterbalanced so to minimise carryover effects and guarantee a sufficient washout of the effects of the previous session. Still, as suggested by Nitsche, Seeber, Frommann, et al. (2005), for tDCS durations that produce short-lasting (namely, for about 10 minutes) after-effects,

a 1-hour break between stimulation sessions is sufficient. However, we agree with the Reviewer that one potential limitation of the study might rely on the repetition of different tDCS conditions (anodal, cathodal, and sham) and the same IAT task within the same day/week. Indeed, it has been previously shown that the magnitude of the IAT effect tends to decline with repeated administrations (Nosek, Greenwald and Banaji, 2007). We have, thus, acknowledged this issue in the limitation section (page 30), where we also further emphasize that the absence of any repetition effects for the control ae-IAT might point against this possibility.

-In the Discussion, the authors argue that cathodal tDCS has more reliable effects than anodal tDCS, however, other consistent literature suggests that anodal tDCS and not cathodal tDCS is more effective. Since the debate is still open on this, and the effects are likely to be highly dependent on the specific function/task assessed, I would tone down a bit the argument that cathodal tDCS is overall more reliable.

Comment No. 3: We thank the Reviewer for this comment and we agree that evidence with regards to the effectiveness of cathodal vs. anodal tDCS is still inconclusive. We have therefore revised the manuscript accordingly. At page 25, we now say: 'However, evidence with regards to the effectiveness of cathodal vs. anodal tDCS is still inconclusive and further experimental manipulations are deemed as necessary to further investigate the potential roles of these factors with respect to the absence of a-tDCS effects over occipital brain areas.'

-The control electrode was placed over the Vertex, in line with prior literature. On the basis of their knowledge on the current flow underlying this specific montage, do the authors think that other areas interested by the current flow may have also contributed to the observed effects?

Comment No. 4: We thank the reviewer for pointing to this important issue. Indeed, we have now expanded the discussion so to include additional remarks on the contribution of this specific montage to the observed effects. Despite the increased use of tDCS and its foreseeable clinical applications, the spatial distribution of the current density within the volume of the human brain for a given electrode montage is largely unknown. A recent study compared the neuroanatomic location and strength of the predicted electric current peaks, at cortical and subcortical levels, induced by conventional and High-Definition-tDCS (HD-tDCS) montages (DaSilva et al., 2015). In particular, by

using a similar montage as the one used in our study (but located on vertex-occipital cortex with anode over Cz and cathode over Oz), authors reported that the visual cortex, cingulate and thalamus received the majority of the current flow. Using this Cz-Oz montage, the current flowed mainly to the parietal and occipital lobes with the maximum electric field occurring in the primary and secondary visual cortices V1/V2. Furthermore, large areas of intense current flow were found in medial neuroanatomical regions such as the inferior peri-insular sulcus (IPS) and posterior insula, bilaterally and in the MCC and posterior cingulate cortex (PCC) on both sides. With this regards, as we are explaining at page 29 of the limitations, we may expect that cathodal stimulation over the left extrastriate visual cortex might have affected nodes of a broader network involved in 'person perception' and 'person knowledge' (e.g., frontal cortex, anterior temporal lobes and the limbic system). It therefore remains to be determined how specific the current results are to the stimulation site and, for example, whether interfering with the activity of the extrastriate visual cortex might have in turn interfered with key areas important for the control of automatic (negative) associations, such as the prefrontal cortices. In a similar vein, we cannot rule out that tDCS may have affected top-down control mechanisms, such as the ability to regulate bias (Conrey et al., 2005) and task-switching abilities (Klauer et al., 2010), that are involved in performing an IAT. Although the gender- and IAT-selectivity of the effects of c-tDCS over left extrastriate visual cortex would speak against general effects on IAT categorization performance, one may speculate that c-tDCS might have affected cognitive control abilities particularly in those individuals (i.e., men) who show higher anti-fat bias and, thus, need more cognitive control to moderate it. Despite the valuable information provided by modelling studies, it is still not possible to precisely define the extent to which the strength of the electric current correlates to the behavioral effects reported with tDCS, as the mechanisms whereby nervous tissue is stimulated by this method are not completely understood. It is certainly undeniable, that adopting in the future a neuroanatomical approach, based for example on computational models, would be crucial in defining the precise neural networks directly modulated by conventional tDCS montages for the study of cognitive processing. Even if we have referred to this issue in the limitation section, we believe that deeper discussion would be beyond the scope of the present paper.

-It would be interesting to extend a bit on how these findings relate to prior studies in which tDCS was used to modulate body image, for instance in patients with anorexia nervosa

Comment No. 5: We are very happy to take on board the Reviewer's suggestion. Accordingly, we have now expanded the discussion by reporting some interesting evidence of the use of non-invasive brain stimulation (NIBS) techniques in patients with Eating Disorders. At page 27, we now say: 'Although, there is currently large evidence to suggest that neuromodulation has potential for altering disordered eating behaviours, food intake and body weight, evidence of using tDCS (and/or TMS) on broader brain network responsible in sustaining ED symptomatology, are still scanty. In fact, much of the research on NIBS and eating behaviour has targeted the dorsomedial and dorsolateral prefrontal cortex (Brass and Haggard, 2007; Campbell-Meiklejohn et al., 2008; Khedr, Elfetoh, Ali, and Noamany, 2014; Kühn et al., 2011; Ljubisavljevic, Maxood, Bjekic, Oommen, & Nagelkerke, 2016; see also McClelland et al., 2013 and Hall & Vincent, 2017 for a recent review on non-invasive brain stimulation for food cravings, consumption, and disorders of eating), which have a key role in self-regulatory control mechanisms (Ochsner & Gross, 2007).

While the prefrontal cortex is very theoretically meaningful as a modulation target for food-related outcomes (Hall, 2016; Miller and Cohen, 2001; Miyake et al., 2000), little attention has been paid to cortical areas that are involved in human visual body processing, such as EBA. Indeed, EBA is generally understood to be necessary for visual body processing and could therefore be a meaningful target for brain modulation. Several studies have shown that EBA is active when subjects are engaged in viewing images of bodies through interconnections with other brain regions, also involved with body image (e.g., ventral premotor cortex; Kitada, Johnsrude, Kochiyama, & Lederman, 2009). Furthermore, Suchan and colleagues (2013), using an fMRI task that showed body images in contrast with images of chairs, found a reduced connectivity between middle occipital gyrus and fusiform body area (FBA) and between FBA and EBA in patients with AN. Some studies have shown that EBA is also activated by the selective display of images of bodies that express emotions (anger, disgust, happiness, fear), supporting a close correlation between extrastriate visual areas and the amygdala, which is involved in processing emotional information (Myers & Sowden, 2008). Furthermore, modulating neural activity of EBA with repetitive transcranial magnetic stimulation altered the hedonic value attributed to body figures by healthy individuals

(Cazzato et al., 2014; 2016). In keeping with this view, our study documents the involvement of these areas in weight-related stereotypes about other individuals. Thus, brain stimulation studies targeting EBA and other relevant body image brain regions may open new horizons to understand the neural substrate of EDs and evaluate the therapeutic potential of tDCS for treating distortions of perception, conceptions and affects related to one's body weight or shape.'

Reviewer #2: This study investigates if anodal or cathodal tDCS over the Extrastriate Body Area (EBA), a region critical for body-shape processing, modulate implicit weight bias as measured by the Implicit Association Test (IAT). Participants completed two versions of the IAT: in one version, they had to associate "FAT" and "SLIM" models with "good" and "bad" concepts, and in the other version with "Ugly" and "Beauty" concepts. This is an interesting and well-designed study exploring the neural correlates of negative attitudes towards overweight individuals. I am generally favourable to the publication of the manuscript but do have some concerns, mostly related to the discussion of findings, that should be addressed.

Comment No. 1: We thank the reviewer for his/her positive evaluation of our paper and for his/her insightful queries and helpful suggestions. We believe that the comments have targeted important areas that required improvement to enhance the overall presentation and clarity of the manuscript.

Abstract/Introduction

I disagree with the authors when they describe the v-IAT as measuring "implicit weight stereotypical associations". Indeed, it seems to me that the main difference between the two IATs is that while the v-IAT is measures general evaluative attitudes towards overweight individuals, the ae-IAT refers to more specific stereotypes of "FAT-ugly". Thus, I would suggest the authors to revise the manuscript accordingly.

Comment No. 2: We thank the reviewer for prompting us to further clarify this issue. We agree that while the valence-IAT aims to measure general (negative) evaluative attitudes towards overweight people, on the contrary the aesthetic-IAT refers to a more specific stereotype of 'FAT-ugly'. Indeed, the aesthetic-IAT was designed as a control condition to focus on body-concepts related to aesthetic dimensions (i.e. "Ugly" and

“Beauty”). At page 7, we have now made this distinction even clearer with the idea of stressing the difference between the two facets of IATs.

Page 6, lines 19-25. The authors should briefly explain what they call the "core person perception network" and how it informs predictions of "selectively modulating the associations between implicit personality judgments and weight-bias". In particular, they should discuss if and in which way it may inform on predictions of differential effects on the v-IAT and ae-IAT.

Comment No. 3: This is a very interesting point and we are grateful to the Reviewer for raising it. At page 7, we know say: ‘In two separated sessions, we applied anodal- (a-), cathodal (c-), or sham-tDCS over the extrastriate visual cortex in the right and left hemispheres of male and female participants with the aim of investigating its role in mediating implicit negative weight stereotypical associations (i.e. ‘bad’ with overweight and ‘good’ with slim) as measured with a weight-related valence-IAT (v-IAT). Furthermore, an ad-hoc IAT, which focused on perceptual dimensions related to body aesthetics (i.e. ‘ugly’ with overweight and ‘beautiful’ with slim), was developed as a control task (aesthetic-IAT, ae-IAT). Importantly, while the v-IAT aimed at measuring general evaluative attitudes towards overweight individuals, the a-IAT referred to a more specific stereotype of ‘FAT-ugly’, which is more related to a perceptual rather than conceptual dimension.’ Furthermore, we have further specified how this model can inform predictions in our task and say, on page 8: ‘In line with Greven, Downing, and Ramsey (2016), Greven and Ramsey (2017) and Quadflieg et al. (2015), we expected that neural activity in extrastriate visual cortex (and particularly in EBA) should provide information about bodily appearance to person knowledge areas (Gobbini and Haxby, 2007; Weiner and Grill-Spector, 2010 and Greven et al., 2016), thus selectively modulating the associations between implicit personality judgments and weight-bias. Conversely, the effects of EBA stimulation are expected to be more limited on the association between two perceptual dimensions of body appearance, namely thinness and beauty, which do not require access to person-specific processing.’

Results

Page 14. Even if effects were specific to c-TDCS the overall statistics on a-TDCS should be reported.

Page 15, line 4. For consistency report the p-value

Please report statistics consistently throughout. Estimated effect sizes are reported only to same F tests. Moreover, non-significant F values are sometimes followed by p-values and other times only by estimated effect sizes.

Comment No. 4: We overlooked this information in the former version of the MS and we thank the reviewer for noticing it. According to his/her suggestion, the overall statistics on a-tDCS, all effect sizes and p-values have been added so that statistics are now reported consistently throughout the Results section.

Discussion

I would like to see more discussion on the differences between v-IAT and ae-IAT and what the present results tell us in light of such differences. I think this is a crucial aspect of the findings but little attention is given to it.

Comment No. 5: This is a very interesting point and we are grateful to the Reviewer for raising it. At page 24, we now say: 'It is worth noting that, while EBA c-tDCS significantly modulated the association between a specific perceptual dimension of the body (i.e., thinness) and general conceptual attributes of a person (i.e., honest, kind etc.), no effects were found on the association between the same perceptual dimension and an evaluative dimension (i.e., aesthetics) related to body perception, but not involving person-specific processing. Thus, EBA c-tDCS did not alter how thin or round bodies appeared or how beautiful they were judged. Its effects were rather specific when body perception involved forming representations about high-level traits of a person. Previous studies (Calvo-Merino et al., 2010; Cazzato et al., 2014, 2016) have shown that magnetic stimulation of EBA alters the judgements of how much an observer likes other people's bodies. These judgements require using basic perceptual aspects, either static (i.e., thinness) or dynamic (posture and movement, Cazzato et al., 2012), to express a general evaluation about the appeal of an unfamiliar individual. Thus, these findings are in keeping with the suggestion (Greven et al., 2016; Greven and Ramsey, 2017; Quadflieg et al., 2015) that body perception processing in EBA (and other body specific areas in the occipito-temporal cortex) is functionally coupled with processing in the theory-of-mind network to form an integrated representation of other people.'

Page 16 line 55 "evident at sham condition". TDCS was not used in the cited studies. I understand that sham is seen as a baseline condition "as if no brain stimulation was applied" but "sham condition" should only refer to situations in which brain stimulation was applied.

Comment No. 6: We agree with the reviewer that sham condition refers to our brain stimulation technique. Accordingly, to avoid any confusion we have now amended the sentence as follows: 'In keeping with the results of previous behavioral studies (Puhl, Luedicke, and Heuer, 2011; Musher- Eizenman, and Carels, 2009), our brain stimulation study found dominant implicit representations of obese individuals as dishonest, villain and immoral when sham stimulation was applied.'

Perhaps the most puzzling aspect of the study is the absence of bias on the v-IAT in female participants. I understand it is not easy to know why, given that this is thought to be a rather consistent bias. Given the relative small sample size it is possible that these specific participants did not have particularly strong general attitudes against overweight individuals. This somewhat diminishes the strength of the findings, but the observation of reduction of bias in males seem consistent enough to sustain the authors claims.

Comment No. 7: We agree with the reviewer that the absence of reliable v-IAT in our female participants deserves discussion. Even if limited sample size might contribute to his absence of effects, our results are in keeping with studies showing greater bias in men than in women. On page 22, we now say: 'The weight v-IAT effect, however, was only significant in male but not in female participants, suggesting a lack of implicit anti-fat bias in women even if no differences were found between men and women in their explicit fat phobic attitudes. Nevertheless, the absence of a significant implicit weight bias in female participants allowed for an indirect control for general effects of tDCS on the IAT performance in the absence of any reliable weight bias. Importantly, this result seems to be in agreement with previous experimental evidence suggesting a strong prevalence of negative attitudes towards overweight individuals and, in general, of social stigma in men as compared to women (Lewis, Cash and Bubb-Lewis, 1997). Most importantly, gender differences in obesity stigma may reflect different conceptions and attitudes toward obesity in the two genders: women usually report significantly greater fear of becoming fat than men do; in contrast, men are significantly more likely to attribute obesity to a lack of willpower and to report greater dislike of obese individuals as compared to women. This is true even after controlling for BMI (Lieberman, Tybur, and Latner, 2012). Hence, future studies should take into consideration specific subtypes of anti-obesity attitudes that may show systematic sex differences, as this is particularly important for future intervention implications (Kelly and Jorm, 2007).'

Page 17 lines 15-23. I don't think these studies (between race differences in obesity bias, or bias against mental illnesses) are relevant for the argument. I encourage the authors to remove these sentences.

Comment No. 8: At page 17, we have now removed these two studies as per the reviewer's suggestions.

Page 17 line 41-42. "anti-fat bias requires the contribution of this area". This study does not provide evidence for the claim that EVC is necessary for weight-bias. Please tone down.

Comment No. 9: In agreement with the Reviewer, we have toned down the sentence to sound as: 'Importantly, after c-tDCS over left extrastriate visual cortex, the men's negative bias for stereotype-congruent stimuli was reduced, revealing that the anti-fat bias involves the contribution of this brain area.**'**

Page 17 line 44-45 "disruption of the congruency-stereotype association". This is not very clear to me. I encourage the authors to revise it.

Comment No. 10: We apologize for the lack of clarity of this statement, which has now been amended as follows: 'That the inhibition of left extrastriate cortex induced a reduction of the weight-bias is in line with previous evidence about implicit processing of emotional faces (Cecere, Bertini and Ladavas, 2013). This study showed that presenting congruent/emotional vs. incongruent/neutral masked faces facilitated responses to emotional faces. However, inhibiting with c-tDCS the activity in the left occipital cortex suppressed this facilitation. This documents the crucial role of the left occipital cortex in mediating high-order implicit visual processes, such as the emotion congruency effects (Cecere, Bertini and Ladavas, 2013).'

Page 18 line 15. Should "such us" be "such as"?

Comment No. 11: Thanks for noticing the typo, which has now been amended accordingly.

Schupp and Renner (2011, Frontiers Human Neuroscience) found modulation of brain activity in occipital-temporal regions in response to images of obese bodies. Such evidence could be acknowledged and discussed.

Comment No. 12: We thank the Reviewer for this suggestion. We have now included and discussed this reference at page 5 and page 27of the Ms. In particular, we believe that Schupp and Renner (2011)' study is especially relevant to ours in that schematic portrays of underweight, normal weight, and overweight body shapes, as well as pictures of tools, were presented to participants with the aim of investigating the neural bases of implicit anti-fat bias by means of event-related potential (ERP) recordings. Indeed, their findings are in accordance with those showing that an early differential ERP activity may be associated with the emotional processing of pictures, faces and words (Wieser et al., 2010) and suggest that the perception of images of obese individuals can modulate early perceptual processing areas, reflecting the intrinsic significance of stimuli (Schupp and Renner; 2011; Wieser et al., 2010).

Limitations

Page 20, lines 28-40. The authors argue that TDCS may have had an effect on top-down control mechanisms. While I agree that this cannot be ruled out, it seems unlikely given the absence of effects over the robust bias on the ae-IAT.

Comment No. 13: Thanks for the appropriate observation. At page 30, we have now reconsidered this issue as follows: 'Although the gender- and IAT-selectivity of the effects of c-tDCS over left extrastriate visual cortex would speak against general effects on IAT categorization performance, one may speculate that c-tDCS might have affected cognitive control abilities particularly in those individuals (i.e., men) who show higher anti-fat bias and, thus, need more cognitive control to moderate it.'

Page 20 line 54 to Page 21 line 14. I don't think this is necessarily a limitation. In fact, it may be relevant to explain the different results in the v-IAT ae-IAT. Similarly, on page 21 lines 15-22, it is not clear to me why this is part of the limitations and not discussion. As mentioned above, I believe the discussion should focus more on the role that EVC may have on the evaluative processing of (fat) bodies and how this contributed to the selective effects on the v-IAT and not ae-IAT.

Comment No. 14: As per the reviewer's suggestions, we have now moved the relevant paragraphs from limitations to the discussion's section. Furthermore, we have discussed more extensively the role that EVC may have on the evaluative processing of fat bodies and how this contributed to the selective effects on the v-IAT and not ae-IAT.

Conclusions

Page 21 lines 53-57. It's not clear to me how the argument of interactions between brain networks comes about. Please revise

Comment No. 15: We thank the reviewer for pointing at the lack of clarity of this paragraph. Accordingly, we have now amended it as follows: 'It has been proposed that the primary function of EBA is grounded on visually analysis of the bodies of conspecifics (Urgesi et al., 2004; Downing & Peelen, 2011). However, during this process EBA may exchange signals not only with other brain circuits that represent aspects of another person's physical appearance (person perception), such as body shape and posture (Cazzato et al., 2014b), but also with brain areas (i.e., TPJ and temporal pole) that respond when reasoning about another person's trait-based characteristics (person knowledge) (Greven et al., 2016). In keeping with previous neuroimaging findings (Greven, Downing and Ramsey, 2016; Ewbank et al., 2011; Quadflieg et al., 2011; Zimmermann et al., 2013), the results of our brain stimulation study provide empirical support for this notion and enhance the belief that interactions between specific person perception and person knowledge neural systems underlie social perception abilities.'

Editor's comments:

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1. Figures: The Editors are striving to have more standardization and clarity of figures in papers published in Neuroscience and as such we have developed a set of comments to pass along to authors. In particular:

- create figures using one of three widths, 82, 120 or 174 mm, for single, one and one-half, or two column formats. If you create one and one-half column figures, Neuroscience now uses text wrapping around the figure and its caption.

- across figures, consistently use only Arial or Helvetica fonts, rather than Times Roman.

- use font sizes that are easily readable when the figures become sized for the print version, no smaller than 8 point for tick mark labels and legends, 10 or 11 point for axis labels and tick mark labels when there's no axis label, and 14 or 16 point for subplot labels (e.g., "A", "B", etc.). Use only upper-case lettering for subplot labels, without parentheses or punctuation. We do not encourage the use of bold fonts.

Comment No. 1: We have now conformed our results presentation to these editorial requirements.

Highlights:

- Attitudes towards slim/obese individuals were measured by means of valence- and aesthetic-IATs
- The role of extrastriate visual cortex in triggering weight stigma associations was investigated
- Cathodal stimulation over left extrastriate visual cortex reduced weight-bias for the valence-IAT only in male participants
- Men’s attitudes towards obese individuals may depend on neural signals from the extrastriate cortex

Title: Cathodal transcranial direct current stimulation of the extrastriate visual cortex modulates implicit anti-fat bias in male, but not female, participants

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Abstract

Explicit negative attitudes towards obese individuals are well documented and seem to modulate the activity of perceptual areas, such as the Extrastriate Body Area (EBA) in the lateral occipito-temporal cortex, which is critical for body-shape perception. Nevertheless, it is still unclear whether EBA serves a role in implicit weight-stereotypical bias, thus reflecting stereotypical trait attribution on the basis of perceptual cues. Here, we used an Implicit Association Test (IAT) to investigate whether applying transcranial direct current stimulation (tDCS) over bilateral extrastriate visual cortex reduces pre-existing implicit weight stereotypical associations (i.e. “Bad” with Fat and “Good” with Slim, valence-IAT). Furthermore, an aesthetic-IAT, which focused on body-concepts related to aesthetic dimensions (i.e. “Ugly” and “Beautiful”), was developed as a control condition. Anodal, cathodal, or sham tDCS (2 mA, 10min) over the right and left lateral occipito-temporal (extrastriate visual) cortex was administered to 13 female and 12 male participants, before performing the IATs. Results showed that cathodal stimulation over the left extrastriate visual cortex reduced weight-bias for the general evaluative (Bad vs. Good) but not specific aesthetic (Ugly vs. Beautiful) dimensions as compared to sham stimulation over the same hemisphere. Furthermore, the effect was specific for the polarity and hemisphere of stimulation. Importantly, tDCS affected the responses only in male participants, who presented a reliable weight-bias during sham condition, but not in female participants, who did not show reliable weight-bias at sham condition. The present results suggest that negative attitudes towards obese individuals may reflect neural signals from the extrastriate visual cortex.

Keywords: anti-fat bias; Extrastriate visual cortex; tDCS; Implicit Association Test

Introduction

There is mounting research evidence that overweight and obese people experience social disadvantages in a multitude of social settings, such as interpersonal relationships, employment, education and healthcare (Puhl and Brownell, 2001; Schupp and Renner, 2011). Indeed, various explicit measures have revealed that being overweight or obese is usually associated with a range of negative features, such as being unattractive, weak-willed and sexually estranged (Crandall, 1994; Phillisp and Hill, 1998; Todorov and Uleman, 2003; Todorov et al., 2008). Furthermore, those negative attitudes towards obese individuals (anti-fat bias) seem to develop in early childhood and they have been even observed in children as young as 3 years old, gradually increasing after that (Cramer and Steinwert, 1998).

More recently, anti-fat bias has been detected (Teachman et al., 2003; Ahern and Hetherington, 2006; Schwartz et al., 2006) by applying “implicit” measures, such as the Implicit Association Test (IAT; Greenwald, Nosek and Banaji, 2003), which can provide an index of the automatic association between the face and body of an obese or slim individual and an evaluative dimension (e.g., Good vs. Bad). Interestingly, participants have shown higher levels of implicit, as compared to self-report measures of bias, thus suggesting that the IAT can reveal levels of prejudice that may not be otherwise apparent (Wang, Brownell and Wadden, 2004). These implicit negative attitudes toward overweight and/or obese individuals can then trigger a range of discriminative, non-verbal behaviours, for example eye contact and spatial distance. Such immediate negative behaviours may take place in the absence of reflective thinking (Todorov and Uleman, 2003), thus providing a constant source of discrimination elicited by the mere sight of an obese person (Schupp and Renner, 2011).

Human beings naturally rely on fundamental cues, such as race, sex and age, in order to categorize others (Fiske, 1993); however these cues may elicit stereotypes about the groups they represent and, thus, yield person-perception processes (Kunda and Thagard, 1996; Macrae et al., 1994). As such, body shape is an important cue to form impressions of other people on the basis of basic perceptual processing. It is still unclear, however, to what extent body-weight negative stereotypes entail only the activity of high-level brain areas involved in evaluative processing or also modulate the activity of brain regions involved in processing visual information conveyed by body shape. In spite of many studies investigating the underlying neural basis of stereotypical attitudes by administering the IAT (e.g., Cattaneo et al., 2011; Crescentini et al., 2014, 2015; Gallate et al., 2011; Gladwin, den Uyl and Wiers, 2012; Chee et al., 2000), only very few studies have so far used neuroimaging and/or neurophysiological techniques to focus on the neural bases of implicit obesity stigma. A seminal fMRI study of Krendl and colleagues (2006) investigated the neural basis of forming either explicit ("Do you like or dislike this person?") and implicit ("Is this a male or female?") judgments of people having well-established stigmatized conditions, such as obesity. The authors of the study proposed the activation of an extensive neural network, including the amygdala, insula, anterior cingulate, and lateral prefrontal cortex that is involved in the processing of highly negative social stigmas. These brain areas have been shown before to be also involved in responding to aversive stimuli, as well as in modulating inhibition and cognitive control. More recently, Azevedo et al. (2014) reported decreased neural reactivity as a result of observing obese people's pain in areas associated with the representation of sensory and affective-motivational aspects of pain (i.e. bilateral insula, somatosensory cortices and thalamus), revealing diminished resonance with obese people's pain.

In a similar vein, Schupp and Renner (2011) investigated the neural bases of implicit anti-fat bias by means of event-related potential (ERP) recordings. In this study, schematic portrays of underweight, normal weight, and overweight body shapes, as well as pictures of tools, served as stimuli. During a first passive viewing task, participants were asked to simply observe the stimuli, while in a subsequent distraction condition participants were asked to detect a specific tool. The authors reported that observing overweight in comparison to normal-weight or underweight body shapes elicited a positive potential shift over fronto-central sites and a relative negative potential over occipito-temporal regions in a time window from ∼190 to 250 msec. No modulation was reported at later time windows. These findings are in accordance with those showing that an early differential ERP activity may be associated with the emotional processing of pictures, faces and words (Wieser et al., 2010) and suggest that the perception of images of obese individuals can modulate early perceptual processing areas, reflecting the intrinsic significance of stimuli (Schupp and Renner; 2011; Wieser et al., 2010). In line with this view, a recent fMRI study of Quadflieg et al. (2011) investigated whether early perceptual aspects of person construal are sensitive to the individuals' stereotype-related status. The authors found that the presentation of targets that violated stereotypic beliefs (e.g., male hairdressers and female airline pilots) increased neural activity not only in areas dedicated to executive control (i.e., DLPFC), but also in extrastriate areas related to person perception. These findings suggest that stereotypic beliefs modulate the activity of extrastriate areas involved in person percept in the brain.

Interestingly, neuroimaging evidence shows that perceptual signals in the ventral visual stream are linked with person-knowledge processing in the Theory-of-Mind network (Greven et al., 2016; Greven & Ramsey, 2017). Specifically, Greven and Ramsey (2017) have recently demonstrated that parts of the extrastriate cortex (EBA), which is involved in the processing of body shape and posture (Urgesi et al., 2004; Downing and Peelen, 2011), exchange signals with areas involved in mentalising and making inferences about others' thoughts and traits (i.e., temporal pole). These findings supports the notion that brain areas that represent aspects of another person's physical appearance (person perception), such as body shape and posture, are coupled to brain circuits that respond when reasoning about another person's trait-based character (person knowledge) (Greven et al., 2016). **However, the functional significance of the contribution of person-perception areas to high level representations of other people's traits is still unclear**. In particular, previous studies have not provided evidence on how modulation of activity in person-perception areas contributes to the formation and reshaping of social biases.

To address this issue, we applied transcranial direct current stimulation (tDCS), a non-invasive brain-stimulation technique that can interfere with cerebral cortex processes by means of a weak electric current passed between two electrodes (anodal and cathodal) on the scalp. This way, decreased (cathodal) or enhanced (anodal) cortical excitability can be induced. We used tDCS to directly manipulate the cortical excitability of the extrastriate visual cortex, including the extrastriate body area (EBA), which has been shown to respond selectively to photorealistic depictions of whole human bodies or body parts, still images of human bodies or body parts extending to 'stick figures' and silhouettes, in preference to human faces, images of objects parts and scenes (Downing et al., 2001; Candidi et al., 2008; Peelen and Downing, 2007; Urgesi et al., 2007a).

In two separated sessions, we applied anodal- (a-), cathodal (c-), or sham-tDCS over the extrastriate visual cortex in the right and left hemispheres of male and female participants with the aim of investigating its role in **mediating implicit negative weight stereotypical associations (i.e. "bad" with overweight and "good" with slim) as measured with a weight-related valence-IAT (v-IAT). Furthermore, an ad-hoc IAT, which focused on perceptual dimensions related to body aesthetics (i.e. 'ugly' with overweight and 'beautiful' with slim), was developed as a control task (aesthetic-IAT, ae-IAT). Importantly, while the v-IAT aimed at measuring general evaluative attitudes towards overweight individuals, the a-IAT referred to a more specific stereotype of 'FAT-ugly', which is more related to a perceptual rather than conceptual dimension. In particular, in these weight-related IATs, participants were required to classify the body of obese and thin people as Fat and Slim, respectively. In parallel, they were required to classify a series of adjectives along two dimensions (general evaluative, Good vs. Bad, or aesthetic, Beautiful vs. Ugly).** In one (congruent) block, bodies and adjectives were randomly presented, while Slim categorization responses were mapped onto the same response key of Good (or Beautiful) categorization responses, whereas Fat and Bad (or Ugly) shared the same response key. In another (incongruent) block, response mapping was inverted, so that the Fat categorizations were mapped with the Good (or Beautiful) ones and the Thin with the Bad (or Ugly) categorizations. In keeping with previous studies (Teachman et al., 2003; Ahern and Hetherington, 2006; Schwartz et al., 2006), we expected participants to be faster to respond in the first pattern than in the second one, which is taken as evidence of 'anti-fat bias'.

In line with Greven, Downing, and Ramsey (2016), Greven and Ramsey (2017) and Quadflieg et al. (2015), we expected that neural activity in extrastriate visual cortex (and particularly in EBA) should provide information about bodily appearance to person knowledge areas (Gobbini and Haxby, 2007; Weiner and Grill-Spector, 2010 and Greven et al., 2016), thus selectively modulating the associations between implicit personality judgments and weight-bias. Conversely, the effects of EBA stimulation are expected to be more limited on the association between two perceptual dimensions of body appearance, namely thinness and beauty, which do not require access to person-specific processing. Predictions regarding the direction of the after-effects of c- and a-tDCS on occipito-temporal areas should be cautious, as they appear to be task-dependent and are still controversial (Antal, Nitsche, and Paulus, 2006). However, based on the results of Quadflieg et al. (2011), showing increased activity of EBA for stereotype-incongruent depictions of human bodies, we expected that inhibiting excitability of extrastriate visual cortex with c-tDCS should reduce implicit anti-fat bias, whereas facilitating excitability of extrastriate visual cortex with tDCS should increase it. Furthermore, comparing the effects obtained for the two weight-related IATs may allow us to verify whether the role of the extrastriate visual cortex is merely related to the perception of body weight (i.e., with comparable effects of tDCS for the v- and ae-IAT) or reflects higher-level involvement in associating specific evaluative dimensions to body forms (i.e., with selective effects for one IAT). Finally, tDCS effects should be influenced by the interindividual differences in implicit and explicit weight-related stereotypes that are expected between men and women

(Lieberman, Tybur and Latner, 2012), with men reporting more negative general attitudes toward obese individuals than women and, consequentially, specific reduction or increase of implicit anti-fat bias after c- or a- tDCS, respectively.

Methods

Participants

A total of 25 students (13 women, range: 20-29 years old; 12 men, range: 20-28 years old) from the University of Udine, Italy, participated in the experiment in return for course credits. Participants were naïve as to the purpose of the study and information about the experimental hypothesis was provided only during the debrief period, after all the experimental tests were completed. All subjects, but one male and one female, were right-handed as identified by means of a Standard Handedness Inventory (Briggs and Nebes, 1975). They were all native Italian speakers of Caucasian race and they all reported heterosexual orientation. Finally, all participants reported normal or corrected to normal vision, they were in good health, free of psychotropic or any other medication, with no past history of psychiatric or neurological disease and with no contraindication to tDCS (Poreisz et al., 2007). At the end of the experiment, participants filled two questionnaires: 1) the Sociocultural Attitudes Toward Appearance Questionnaire-3 (SATAQ-3; 4 scales; Stefanile et al., 2011; Thompson et al., 2004) to measure multiple aspects of societal influence, such as the degree of mass media internalization of the models; 2) the Fat Phobia scale (short version from Bacon et al., 2001) in order to measure fat phobic attitudes. In particular, The Fat Phobia Scale – short form (Bacon et al., 2001) assesses explicit negative attitudes and stereotyped perceptions of obese people. This scale consists of 14 pairs of adjectives that are sometimes used to describe obese individuals. For each pair, participants have to indicate, using a 5-point scale, the adjective that best describes their feelings and believes (e.g. 1 = Industrious/5 = Lazy). Higher scores reflect greater fat phobia. Furthermore, we estimated participants' BMI from self-report measures of weight (Kg) and height (cm). The participants' demographics and self-report questionnaire scores as a function of gender are reported in Table 1. Participants gave their written informed consent and all experimental procedures were previously approved by the ethics committee of the Scientific Institute (IRCCS) 'E. Medea' and were in accordance with the ethical standards of the Declaration of Helsinki (1964).

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Materials and Methods

Body Stimuli

All participants were shown a series of 6 virtual human models (3 females / 3 males) previously selected from a database of adult body stimuli created by means of Poser Pro 2010 (e-frontier, Santa Cruz, CA) (for details see Cazzato et al., 2012). Virtual models rather than "real" persons were used in order to limit confounds related to differences in attractiveness, clothing, attire, and familiarity **(Schupp and Renner, 2011)**. The coloured virtual models were rendered in two different static daily poses (e.g., standing). The body weight was gradually increased or decreased in order to create two body size extremes for each model (fat/slim). All pictures were taken with the models standing in frontal-view, against a grey background and wearing identical black clothing (underwear). Following that, photorealistic textures were applied and the images were rendered with global illumination. Finally, in order to avoid the influence of any facial features, the pictures were imported into Adobe Photoshop 7.0 (Adobe System Inc. CA; http://www.adobe.com) and a circle region around the face was scrambled.

IAT words

A pilot study was run to appropriately select words stimuli for the valence (good and bad) and aesthetic (beautiful and ugly) categories, which were used respectively in the v-IAT and ae-IAT**.** The entire corpus of evaluative- and aesthetics-related adjectives was selected among a larger sample of words contained in the COLFIS database (CoLFIS database: Corpus and Frequency Lexicon of Written Italian, Bambini and Trevisan, 2012). An independent group of 25 Italian subjects (9 males and 16 females; range: 18-36 years old), who did not take part in the tDCS experiment, rated each word (n=94) on a series of 7-point Likert scale by judging: 1) familiarity (subjective report about how frequently a word occurs in the life of a person); 2) imageability (ease and speed of a word in evoking a mental image or a sensory experience); 3) concreteness (reference to objects, living things, actions and materials that can be experienced through the senses); 4) valence (ability of a word to elicit in the speaker and listener positive or negative feelings) and 5) strength of association of each adjective with aesthetic and valence dimensions. Table 2 reports the mean values for each of the above-mentioned dimensions for the four categories of stimulus words. A total of final forty-eight words (12 for each category) were selected as stimuli (see Table 3). A series of one-way ANOVAs on each dimension indicated that the categories were matched for familiarity [$F(3,44) = 2.130$, $p = 0.110$, $\eta p^2 = 0.127$], imageability [$F(3,44) = 2.540$, $p < 0.069$, $\eta p^2 = 0.148$], length of letters [$F(3,44) = 1.321$, $p = 0.280$, $\eta p^2 = 0.083$] and frequency of word use in Italian language (COLFIS database) [$F(3,44) = 1.145$, $p = 0.341$, $\eta p^2 = 0.072$], but not for concreteness [$F(3,44) = 13.954$, $p < 0.001$, $\eta p^2 = 0.488$]. Newman-Keuls post hoc tests for the concreteness measure showed that the words used in the aesthetic category (Beautiful and Ugly) were judged more concrete than the other two categories of words (Valence: Bad and Good) (all $p < 0.001$). Importantly, the analysis on valence ratings revealed a main effect of category [$F(3,44) = 326.896$, $p < 0.001$, $\eta p^2 = 0.957$], with Beautiful and Good words having more positive valence than the other two types of words (all $p < 0.001$). Finally, the analysis on the strength of association (difference between the association of each word with the aesthetic and valence dimensions) confirmed that Beautiful and Ugly words were more associated with the aesthetic than the valence dimension and that Good and Bad words were more associated with the valence than the aesthetic dimension [$F(3,44) = 42.393$, $p < 0.001$, $\eta p^2 = 0.743$; all $p< 0.001$]. Thus, the pilot experiment confirmed the validity of our measures of aesthetic and valence representations.

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Experimental Procedure

During the experiment, participants were seated in a dimly light room at a distance of approximately 57 cm away from a LCD monitor (19 inches, resolution of 1024*768 pixels, refresh frequency at 60 Hz)**.** The experiment was designed and controlled with E-Prime software (version 2.0 Professional, Psychology Software Tools, Inc., Pittsburgh, PA). At the beginning participants had to complete their demographic details, followed by brief written instructions about the task and, then, by the v-IAT. Participants were instructed to respond as fast and accurate as possible immediately after the onset of the stimuli (i.e., single words or images presented one at a time at the centre of the screen), by pressing a left (E) or a right (I) key on the computer keyboard with the index finger of their left and right hand, respectively. Each IAT lasted approximately 8 minutes and was administered in seven blocks, each consisting of both congruent and incongruent condition blocks (blocks 3, 4, 6, and 7) and familiarization blocks (blocks 1, 2, and 5) (Greenwald, 2003; Cattaneo et al., 2011; Crescentini et al., 2014). Before the first running of each IAT, participants were shown a list with all the words belonging to the two relevant categories and they were asked to carefully study all the stimuli.

In the first block of v-IAT, 12 images of Fat and 12 images of Slim people were presented and had to be classified as being either Fat (left key) or Slim (right key). Each of the 12 images of the two categories was presented only once for a total of 24 trials. The second block also consisted of 24 trials, in which Bad-related (requiring a left-key response) and Good-related (requiring a right-key response) words were presented. In the third block (24 practice trials) and in the fourth block (48 test trials), both Fat and Slim bodies and Good and Bad words were randomly presented and participants were instructed to press the left key for Bad-related words and images of Fat people, and the right key for Good-related words and images of Slim people (congruent-stereotype condition). In the fifth block (24 trials), response key assignments were reversed in relation to the categorization involving images of fat people (right key) and images of slim people (left key). Finally, in the sixth block (24 practice trials) and in the seventh block (48 test trials), both Fat and Slim bodies and Good and Bad words were randomly presented and participants were required to press the left key for images of Fat people and Good words and the right key for images of Slim people and Bad words (incongruent-stereotype condition) (see Table 3). Typically, participants are faster and more accurate in the congruent- than in the incongruent-stereotype blocks, thus demonstrating an automatic association between Fat and Bad categories and Slim and Good categories (Greenwald, Banaji and Nosek, 2003).

With regards to the control ae-IAT, the procedure was exactly the same as the v-IAT, with the exception that aesthetics-related words were presented and participants were instructed to classify the words as being related to Beautiful or Ugly categories (see Table 3). The 12 images of fat and slim people presented during the v-IAT were also used in the ae-IAT. Stimuli within each block were presented in random order. Each stimulus (word/image) persisted on the computer screen until the participant gave a correct response. If participants made an error, then a red "X" appeared below the word stimulus in order to prompt them to correct the mistake and press the correct key. Following the response, the next stimulus appeared after 500 msec, during which only the category labels were visible on the screen. **In two separate days (one per each hemisphere), the two IATs were presented to each participant in three blocks, one for each of the stimulation type (sham, a- and c-tDCS). Each block lasted for about 20 min (tDCS stimulation + task duration). Moreover, half of the participants performed first the**

v-IAT and then the ae-IAT; the opposite order was used for the other half. Finally, after the tDCS experiment, participants were required to provide information about their weight and height (for calculating BMI) and to complete the SATAQ-3 and Fat Phobia Questionnaires.

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tDCS

Anodal, cathodal or sham-tDCS (2 mA) was delivered by means of a battery-driven, constant-current stimulator (BrainStim, EMS, Bologna, Italy) through a pair of saline-soaked sponge electrodes (5 × 5 cm, 25 cm^2).

The electrodes were first firmly attached by elastic bands and saline solution was applied under the electrodes in order to reduce contact impedance before the montage. To comply with current safety regulations (Poreisz et al., 2007), a constant current of 2 mA intensity was applied. Specifically, the stimulating current was ramped up during a 10-sec fade-in phase, then held constant at 2 mA for 10 min, and then ramped down during a 10-sec fade-out phase. We chose this specific duration of the tDCS stimulation on the basis of previously reported experimental protocols, which have described effects on cortical excitability, sufficiently enduring to cover the duration of the experimental task (Nitsche and Paulus, 2001; Mancini et al., 2012). The experimental task was initiated exactly in the last 2 min of tDCS. In each daily session, the participants received a-, c-, and s-tDCS on the same hemisphere in three separate blocks. **The order of the hemisphere daily sessions and of the stimulation-condition blocks was counterbalanced across**

subjects. An interval of 3-5 days was allowed between the two daily sessions and of at least 90 min between the three stimulation-condition blocks in order to avoid carryover effects and to guarantee a sufficient washout of the effects of the previous session (e.g., Mancini et al., 2012; Bolognini, et al., 2010; 2011). During the 90 minutes of break, participants were free to leave the laboratory and take some rest. During the three different experimental blocks, the location of the active electrode was identified by means of the 10–20 system for EEG electrode placement. In keeping with previous studies targeting the lateral occipito-temporal cortex with tDCS (Mancini et al., 2012), the active electrode was placed between O2 and PO8 to stimulate the extrastriate visual cortex, including visual body-specific regions (Mancini et al., 2012; Downing et al., 2001). The reference electrode was always fixed on the vertex (Cz). Moreover, as in previous studies, for the sham condition, the electrodes were placed over the target sites (see Fig. 1), with the same parameters of a- and c-tDCS, but the stimulator was turned off after 30 sec (Nitsche and Paulus, 2000; Mancini et al., 2012). This ensured that participants could initially feel the itching sensation at the beginning of the tDCS protocol, but no effective modulation of cortical excitability could be elicited (Gandiga, Hummel and Cohen, 2006). Finally, in-house software switched the tDCS on and off without intervention from the participants or experimenters, allowing for successful blinding.

-------------------------------- Please insert Fig. 1 around here --------------------------------

Data Handling

Statistical analyses were performed on the mean D-scores, which measure the IAT effects by combining both accuracy and speed aspects of responses and were computed following the improved algorithm procedure described by Greenwald et al. (2003) and Cattaneo et al. (2011). In particular, we first checked that there were no trials with latencies greater than 10,000 msecs and no participants responded faster than 300 msecs in more than 10% of all the experimental trials. Then, for computing the mean reaction times (RTs), RTs of error trials were removed and replaced with the mean RTs of correct trials in the corresponding block plus an addition of 600 msec. To compute D-scores, the mean RTs of block 3 were subtracted from the mean RTs of block 6 and the difference was divided by the pooled SD of all trials in blocks 3 and 6; similarly, the mean RTs of block 4 were subtracted from the mean RTs of block 7 and the difference was divided by the pooled SD of all trials in blocks 4 and 7. Finally, the two quotients obtained in the previous two steps were averaged (Cattaneo et al., 2011). For the sake of clarity, error rates and RTs of correct responses are reported in Table 4, respectively for each IAT.

First, we tested whether male and female participants presented with significant weight bias in the two IATs at the baseline (sham) condition by comparing the corresponding mean D-scores to zero (where zero refers to the absence of any response bias). Then, to test the effects of tDCS on the implicit association of weight to good/bad attributes and to control beautiful/ugly attributes, the D-score data were entered into two separated mixed-model Analyses of Variance (ANOVAs), one for each IAT, with gender group (male, female participants) as between-subjects factor and tDCS stimulation (anodal, cathodal, sham) and Hemisphere (left, right) as within-subject variables. Significant three-way interactions were followed up by separate 2-way ANOVAs in each gender group, while the source of significant two-way interactions was analysed using the Newman-Keuls post-hoc test.

Finally, we calculated, for each condition, a measure of the change of v-IAT D-scores as the difference between the individual values after c- and a-tDCS and the corresponding values in the sham-tDCS condition [active-tDCS − sham-tDCS]. The change indexes were correlated, using Pearson correlations, with BMI and individual scores at the Fat Phobia Scale and SATAQ questionnaire.

All statistical analyses were performed with STATISTICA 8.0 (StatSoft Inc, Tulsa, Oklahoma). Effect sizes were estimated using the partial eta square variable (η_p^2). All data are reported as Mean (M) and Standard Error of the Mean (s.e.m.). A significance threshold of $p < 0.05$ was set for all effects.

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Results

Valence-IAT

One sample t-tests comparing the mean D-scores to zero showed that male participants showed a significant stereotypical anti-fat bias in both sham-tDCS conditions, indicating that they were more prone to associate fat people to the bad-related category and slim people to the good-related category than vice versa [$t(11) = 3.56$, $p = 0.004$ for right sham-tDCS and $t(11) = 5.04$, $p < 0.001$ for left sham-tDCS]. Conversely, the analysis of the female participants' mean D-scores revealed absence of the anti-fat bias in both sham-tDCS conditions, namely for right [$t(12) = 1.15$, $p = 0.271$] and for left sham-tDCS [$t(12) = 1.61$, $p = 0.134$].

The 3-way ANOVA on the v-IAT revealed a significant 3-way interaction of hemisphere × tDCS stimulations × gender [$F_{(2,46)} = 3.356$; $p = 0.044$; $\eta_p^2 = 0.127$]. The follow-up 2 × 3 ANOVA on the mean D-scores for male participants revealed a significant *2*-way interaction of hemisphere × tDCS stimulations [$F_{(2,22)} = 7.522$; $p = 0.003$; $\eta_p^2 = 0.406$], but no main effects of hemisphere [$F = 0.794$, $p = 0.392$; $\eta_p^2 = 0.067$] or stimulation [$F = 0.924$, $p = 0.412$; $\eta_p^2 = 0.077$]. Newman-Keuls post-hoc comparisons showed that c-tDCS over left extrastriate visual cortex reduced the weight-bias for the v-IAT, as compared to sham [0.13 ± 0.7 vs. 0.44 ± 0.09, $p = 0.007$]. The effect was specific for the polarity and hemisphere of stimulation, since the weight-bias after c-tDCS over the left extrastriate visual cortex was significantly lower than that after c-tDCS over the right extrastriate visual cortex [0.13 ± 0.7 vs. 0.37 ± 0.06; $p = 0.035$; see Fig. 2A]. Crucially, the difference between the two sham conditions in the right and left hemisphere stimulation sessions was not statistically significant [0.26 ± 0.07 vs. 0.44 ± 0.09; p = 0.126]. Furthermore, the difference between a-tDCS over extrastriate visual cortex as compared to the relative sham condition was not statistically different for both right [0.30 ± 0.06 vs. 0.26 ± 0.07, $p = 0.568$] and left [0.23 ± 0.08 vs. 0.44 ± 0.09, $p = 0.096$] hemispheres. Finally, non-significant difference was observed between right and left a-tDCS conditions [$p = 0.637$].

-------------------------------- Please insert Fig. 2 around here --------------------------------

The 2 ×3 ANOVA on the mean D-scores of female participants revealed non-significant main effects of hemisphere and stimulation and non-significant interaction [all *Fs* < 1.367, all *ps* > 0.274; all η_p^2< 0.102] (See Fig. 2B).

Aesthetic-IAT

At baseline, male participants showed a significant stereotypical anti-fat bias in both sham-tDCS conditions, indicating that they were more prone to associate fat people to the ugly-related category and slim people to the beautiful-related category than vice versa [*t*(11) = 0.29, *p* = 0.007 for right sham-tDCS and *t*(11) = 0.40, *p* < 0.001 for left sham-tDCS]. The analysis of the female participants' mean D-scores revealed a significant anti-fat bias in both sham-tDCS conditions, namely for right [*t*(12) = 0.3, *p* = 0.015] and for left sham-tDCS [*t*(12) = 0.34, *p* = 0.010]. Thus, the aesthetic anti-fat bias was apparent in both gender groups.

However, the 3-way ANOVA on the ae-IAT D-scores (Fig. 3) revealed non-significant main effects or interactions *[all Fs* < *0.724;* all *ps* > 0.404; η_p^2 < 0.031]. In particular, the non-significant 3-way interaction between gender group, hemisphere, and stimulation [$F_{(2,46)}$ = 0.199*; p = 0.821;* η_p^2 = 0.009] suggests that the gender- and hemisphere- specific modulation of the weight-bias in the valence dimension was not reflected in the aesthetic dimension.

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Self-reported questionnaires

As shown in Table 1, independent sample t-tests indicated that male and female participants were matched for both age and BMI. The analysis of the SATAQ-3 data revealed that, compared to women, men had higher scores on the internalization-athlete SATAQ-3 subscale, thus indicating that they might have a stronger internalization of media influences related to the achievement of an athletic physique (Internalization-Athlete); conversely, the two gender groups did not differ on the thin-ideal internalization score (Internalization-General), the perceived feelings of pressure to conform to the Western ideals exhibited by the media (Pressures) and the recognition of the social importance of the media's messages about Western beauty ideals information (Information). Furthermore, no differences were found between men and women in the explicit phobic attitude towards fat people. Finally, no significant correlations were found between the tDCS change indexes and the BMI, Fat Phobia, and SATAQ subscales for both male and female participants ($-0.612 <$ all $rs < 0.433$).

Discussion

This study applied tDCS to examine whether non-invasive brain stimulation can modulate anti-fat bias, and we demonstrated that stimulation over the left, but not right, extrastriate visual cortex, where EBA has been previously located (Sadeh et al., 2011; Taylor et al., 2010), decreased negative attitude towards fat people. Importantly, we also developed a control ae-IAT, which focused on body-concepts related to aesthetic representations (i.e. "ugly" and "beauty") and we found that inhibiting neural excitability in the left occipital cortex by applying c-tDCS diminished the anti-fat bias only for the v-

IAT but not for the ae-IAT. Conversely, enhancing cortical excitability through a-tDCS did not exert any effects in either hemisphere. Interestingly, the effects of tDCS for the v-IAT were found only in male participants, who displayed a significant anti-fat bias, but not in female participants, who did not show a reliable anti-fat bias. To the best of our knowledge this is the first study showing a causative role of the lateral occipito-temporal cortex in the anti-fat bias.

In keeping with the results of previous behavioral studies (Puhl, Luedicke, and Heuer, 2011; Musher-Eizenman, and Carels, 2009), our brain stimulation study found dominant implicit representations of obese individuals as dishonest, villain and immoral when sham stimulation was applied. The weight v-IAT effect, however, was only significant in male but not in female participants, suggesting a lack of implicit anti-fat bias in women even if no differences were found between men and women in their explicit fat phobic attitudes. Nevertheless, the absence of a significant implicit weight bias in female participants allowed for an indirect control for general effects of tDCS on the IAT performance in the absence of any reliable weight bias. Importantly, this result seems to be in agreement with previous experimental evidence suggesting a strong prevalence of negative attitudes towards overweight individuals and, in general, of social stigma in men as compared to women (Lewis, Cash and Bubb-Lewis, 1997). Most importantly, gender differences in obesity stigma may reflect different conceptions and attitudes toward obesity in the two genders: women usually report significantly greater fear of becoming fat than men do; in contrast, men are significantly more likely to attribute obesity to a lack of willpower and to report greater dislike of obese individuals as compared to women. This is true even after controlling for BMI (Lieberman, Tybur, and Latner,

2012). Hence, future studies should take into consideration specific subtypes of anti-obesity attitudes that may show systematic sex differences, as this is particularly important for future intervention implications (Kelly, Jorm and Wright, 2007).

Importantly, after c-tDCS over left extrastriate visual cortex, the men's negative bias for stereotype-congruent stimuli was reduced, revealing that the anti-fat bias involves the contribution of this brain area. That the inhibition of left extrastriate cortex induced a reduction of the weight-bias is in line with previous evidence about implicit processing of emotional faces (Cecere, Bertini and Ladavas, 2013). This study showed that presenting congruent/emotional vs. incongruent/neutral masked faces facilitated responses to emotional faces. However, inhibiting with c-tDCS the activity in the left occipital cortex suppressed this facilitation. This documents the crucial role of the left occipital cortex in mediating high-order implicit visual processes, such as the emotion congruency effects (Cecere, Bertini and Ladavas, 2013).

It has been previously shown that the extrastriate visual cortex and the functional localized EBA is causatively involved in mapping morphological features of human bodies (Downing et al., 2001; Candidi et al., 2008; Urgesi et al., 2007a). This process can prove critical for maintaining constant the identity of others, even when body configurations change drastically during action sequences. Thus, the role of EBA may be fundamental for the identification of actors, particularly when facial cues are unavailable or ambiguous. Indeed, several studies have shown that EBA is sensitive to subtle variations of human body size and shape (Aleong and Paus, 2010) in healthy individuals and its neuro-functional alteration is associated with body image disturbance, such as body size overestimation and negative evaluation of one's own body, in patients with Eating Disorders (ED) (Uher et al., 2005). The present study shows that neural activity in the extrastriate visual cortex, and possibly EBA, may play a role in contributing to implicit weight-stereotypical bias. This may reflect top-down modulation due, for example, to increased attention towards fat as compared to thin bodies. Hence, our results extended previous knowledge (e.g., Quadflieg et al., 2011) on the role of perceptual processing areas in social biases by showing that artificially modulating the neural excitability of extrastriate visual areas implicated in the evaluation of body shape (Urgesi et al., 2007b; Downing et al., 2001) can change prejudice towards fat people.

It is worth noting that, while EBA c-tDCS significantly modulated the association between a specific perceptual dimension of the body (i.e., thinness) and general conceptual attributes of a person (i.e., honest, kind etc.), no effects were found on the association between the same perceptual dimension and an evaluative dimension (i.e., aesthetics) related to body perception, but not involving person-specific processing. Thus, EBA c-tDCS did not alter how thin or round bodies appeared or how beautiful they were judged. Its effects were rather specific when body perception involved forming representations about high-level traits of a person. Previous studies (Calvo-Merino et al., 2010; Cazzato et al., 2014a, 2016a) have shown that magnetic stimulation of EBA alters the judgements of how much an observer likes other people's bodies. These judgements require using basic perceptual aspects, either static (i.e., thinness) or dynamic (Cazzato et al., 2012), to express a general evaluation about the appeal of an unfamiliar individual. Thus, these findings are in keeping with the suggestion (Greven et al., 2016; Greven and Ramsey, 2017; Quadflieg et al., 2015) that body perception processing in EBA (and

other body specific areas in the occipito-temporal cortex) is functionally coupled with person knowledge processing in the theory-of-mind network to form an integrated representation of other people.

In spite of the reliable effects of EBA c-TDCS, a-tDCS of the extrastriate visual cortex did not modulate the anti-fat bias. Anodal-tDCS has been shown to enhance perceptual (Falcone et al., 2007) and motor (Nitsche et al., 2003) learning, social ability (Santiesteban et al., 2012), and visual analgesia (Mancini et al., 2012). However, studies using tDCS in animal models (Bindman et al., 1964; Creutzfeldt, Fromm and Kapp, 1962) have shown that the effect of cathodal stimulation may be stronger than the effect of anodal stimulation if identical stimulation parameters are used. This is in line with the general observation of asymmetric neuroplastic effects in the central nervous system, with excitability reductions being easier to elicit than excitability increases, as shown in animals in vivo (Froc et al., 2000; see Antal et al. 2006 for a review on tDCS effects on visual cortex). Part of the explanation of this asymmetry may reside in the fact that in some experiments the visual system is probably already optimally tuned in healthy subjects and, thus, an excitatory enhancement induced by a-tDCS cannot further improve the perception of visual stimuli (Antal et al., 2006). **However, evidence with regards to the effectiveness of cathodal vs. anodal tDCS is still inconclusive and further experimental manipulations are deemed as necessary to further investigate the potential roles of these factors with respect to the absence of a-tDCS effects over occipital brain areas.**

Overall, these findings support the notion that additional factors, such as the orientation of the electric field (e.g., Nitsche and Paulus, 2000) and the background level of activity in the system when tDCS is applied, might have affected our results. Hence, some features of the task-related activation may interact with the physiological state of the cortex and polarity of tDCS stimulation (Vallar and Bolognini, 2011; Antal and Paulus, 2008; Antal et al., 2004).

A further result of the present study is that, despite differences between the two gender groups, no relation was observed between the changes of weight bias after c-tDCS and the individual level of explicit phobic attitude and internalization of Western ideals and BMI. This might be due to the fact that the range of observers' BMI and self-report measures within our female and male samples was not large enough to disclose any relevant effects of interindividual differences. This finding, however, is in keeping with a study of Teachman and Brownell (2001) and Teachman et al. (2003), who found no evidence of statistically significant relation between the Fat Phobia Scale and implicit bias as detected with a bad/good weight-IAT that was similar to our task. Such dissociation between implicit and explicit measures of anti-fat bias might result from considering social undesirable the labelling of obese individuals as 'bad' (Teachman and Brownell, 2001).

The possible mediating role of perceived attractiveness of the body stimuli used during both IATs needs to be considered. Indeed, some researchers have claimed that anti-fat prejudice may stem from the perception of overweight individuals as unattractive or aesthetically displeasing (e.g., Morrison and O'Connor, 1999). However, we found gender differences in the v-IAT during sham stimulation, but both male and female participants showed reliable implicit weight-bias in the association of fat or slim bodies to the beautiful-ugly dimension in the ae-IAT. Furthermore, tDCS affected men's v-IAT, but no specific tDCS modulation was found for the ae-IAT, suggesting that valence and aesthetic evaluations may be two independent judgement categories during person perception and might be underpinned by different neural circuitry. Furthermore, during the IAT procedure, participants are explicitly required to classify stimuli according to their body weight. **Thus, it is unclear whether body-related perceptual areas are similarly involved when anti-fat bias is prompted by the mere sight of an obese body independently from explicit focus on the weight dimension (Moors and De Houwer, 2006; Schupp and Renner, 2011; see also Quadflieg et al., 2011).**

The present findings might have clinical relevance for the understanding and treatment of body schema disturbances in Eating Disorders (EDs). Although, there is currently large evidence to suggest that neuromodulation has potential for altering disordered eating behaviours, food intake and body weight, evidence of using tDCS (and/or TMS) on broader brain network responsible in sustaining ED symptomatology, are still scanty. In fact, much of the research on neuromodulation and eating behaviour has targeted the dorsomedial and dorsolateral prefrontal cortex (Brass and Haggard, 2007; Campbell-Meiklejohn et al., 2008; Khedr, Elfetoh, Ali, and Noamany, 2014; Ljubisavljevic, Maxood, Bjekic, Oommen, & Nagelkerke, 2016; see also McClelland et al., 2013 and Hall & Vincent, 2017 for a recent review on non-invasive brain stimulation for food cravings, consumption, and disorders of eating), which have a key role in self-regulatory control mechanisms (Ochsner & Gross, 2008).

While the prefrontal cortex is very theoretically meaningful as a modulation target for food-related outcomes (Miller and Cohen, 2001), little attention has been paid to cortical areas that are involved in human visual body processing. Indeed, recent studies have shown that perceptual adaptation to model bodies may alter weight-related body preferences in healthy individuals and patients with EDs

(Winkler and Rhodes, 2005; Glauert et al., 2009; Mele et al., 2013, 2016; Cazzato et al., 2016b).

Importantly, several studies have shown that EBA is active when subjects are engaged in viewing images of bodies through interconnections with other brain regions, also involved with body image (e.g., ventral premotor cortex; Kitada, Johnsrude, Kochiyama, & Lederman, 2009). Furthermore, Suchan and colleagues (2013), using an fMRI task that showed body images in contrast with images of chairs, found a reduced connectivity between middle occipital gyrus and fusiform body area (FBA) and between FBA and EBA in patients with AN. Some studies have shown that EBA is also activated by the selective display of images of bodies that express emotions (anger, disgust, happiness, fear), supporting a close correlation between extrastriate visual areas and the amygdala, which is involved in processing emotional information (Myers & Sowden, 2008). Furthermore, modulating neural activity of EBA with repetitive transcranial magnetic stimulation altered the hedonic value attributed to body figures by healthy individuals (Cazzato et al., 2014; 2016). In keeping with this view, our study documents the involvement of these areas in weight-related stereotypes about other individuals. Thus, brain stimulation studies targeting EBA and other relevant body image brain regions may open new horizons to understand the neural substrate of EDs and evaluate the therapeutic potential of tDCS for treating distortions of perception, conceptions and affects related to one's body weight or shape.

Limitations

There are a few limitations to consider when interpreting the current findings. First of all, we need to consider that the spatial resolution of tDCS, due to using large sponge pads positioned on the skull, can be relatively diffuse. **Indeed, it has been previously reported that brain stimulation by means of tDCS protocols is unlikely to be constrained to the cortex underneath the electrodes (Datta et al., 2009; Bikson and Rahman, 2013; Bestmann, de Berker and Bonaiuto, 2015). In particular, a recent modelling study (DaSilva et al., 2015) estimated that, with a similar vertex-occipital cortex montage (with the anode over Cz and cathode over Oz), current flows mainly to the parietal and occipital lobes with the maximum electric field occurring in the primary and secondary visual cortices. However, current flow extended to the cingulate cortex, insula, central sulcus and thalamus. As such, we cannot rule out that that stimulation of extrastriate visual cortex might have affected nodes of a broader network involved in person perception and person knowledge. Indeed, it has been previously reported that the frontal cortex, anterior temporal lobes and the limbic system are key areas implicated in the forming of social prejudice.** More specifically, the amygdala has been found to be critically involved in cognitive and affective learning, including implicit attitudes (Amodio and Devine, 2006; Dolan et al., 2000; Phelps, Cannistraci, and Cunningham, 2003; Stanley, Phelps, and Banaji, 2008). Furthermore, recent experimental evidence has proposed a critical involvement of the anterior temporal lobes in expressing prejudice by means of conceptual processing (Snyder, Bossomaier, and Mitchell, 2004; Gallate et al., 2011). Finally, a study of Cattaneo and colleagues (2011) demonstrated the causal role of the prefrontal cortex in controlling gender stereotypical beliefs in men. Interestingly, they found that non-invasive brain stimulation delivered at stimulus presentation over the prefrontal cortices led to an increased gender-stereotypical bias for the D-scores of male participants, as compared to a control condition. It therefore remains to be determined how specific the current results are to the stimulation site and, for example, whether interfering with the activity of the extrastriate visual cortex might have in turn interfered with key areas important for the control of automatic (negative) associations, such as the prefrontal cortices.

In a similar vein, we cannot rule out that tDCS may have affected top-down control mechanisms, such as the ability to regulate bias (Conrey et al., 2005) and task-switching abilities (Klauer et al., 2010), which are involved in performing an IAT. **Although the gender- and IAT-selectivity of the effects of c-tDCS over left extrastriate visual cortex would speak against general effects on IAT categorization performance, one may speculate that c-tDCS might have affected cognitive control abilities particularly in those individuals (i.e., men) who show higher anti-fat bias and, thus, need more cognitive control to moderate it.**

Although the order of testing was counterbalanced across participants, one potential limitation of this study could rely on the repetition of the same IAT task under different tDCS conditions (anodal, cathodal, sham) within the same day/week. Indeed, it has been shown that the magnitude of the effect tends to decline with repeated administrations (Nosek, Greenwald and Banaji, 2007). However, the absence of any repetition effects for the control ae-IAT points against this possibility.

Finally, it cannot be determined to what extent the selective decrease in the anti-fat bias after EBA c-tDCS observed in this study can be generalised to other specific subtypes of anti-obesity attitudes and/or social stigma in general. Further studies are required to systematically examine the effects of tDCS on various negative attitudes against stigmatized social groups.

Conclusions

Overall, the present study may contribute to the growing social neuroscience literature on the neural underpinnings of person perception, thus extending previously reported work on explicit and implicit weight stigma as a function of first impression formation (e.g. facial attractiveness, trustworthiness, and competence). Previous neuroimaging studies (e.g., Quadflieg et al., 2011) have shown that early perceptual aspects of person construal are sensitive to the stereotype-related status of individuals. Here, we provided causative evidence that activity in body-selective occipito-temporal areas actively contributes to the formation and expression of implicit stigma based on body size. This pairing of functional responses between distinct brain circuits may indicate that person-perception and person-knowledge neural networks are not entirely encapsulated from other neural brain systems. **It has been proposed that the primary function of EBA is grounded on visually analysis of the bodies of conspecifics (Urgesi et al., 2004; Downing & Peelen, 2011). However, during this process EBA may exchange signals not only with other brain circuits that represent aspects of another person's physical appearance (person perception), such as body shape and posture (Cazzato et al., 2014), but also with brain areas (i.e., TPJ and temporal pole) that respond when reasoning about another person's trait-based characteristics (person knowledge) (Greven et al., 2017). In keeping with previous neuroimaging findings (Greven, Downing and Ramsey, 2016; Ewbank et al., 2011; Quadflieg et al., 2011;**

Zimmermann et al., 2013), the results of our brain stimulation study provide empirical support for this notion and enhance the belief that interactions between specific person perception and person knowledge neural systems underlie social perception abilities.

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Figures Legends:

Fig. 1: Schematic representation of tDCS electrodes montage over left and right Extrastriate visual cortex.

Fig. 2: Effects of cathodal (c-tDCS), anodal (a-tDCS) and sham-tDCS (s-tDCS) on D-scores as a function of gender (men, women) and t-DCS hemisphere (right EVC, left EVC) for the valence-IAT. A: male participants, B: female participants. *Error bars* indicate standard errors mean over participants * $p < 0.05$. Notes: tDCS. Transcranial direct current stimulation; EVC. Extrastriate Visual Cortex; IAT. Implicit association test.

Fig. 3: Effects of cathodal (c-tDCS), anodal (a-tDCS) and sham-tDCS (s-tDCS) on D-scores as a function of gender (men, women) and t-DCS hemisphere (right EVC, left EVC) for the aesthetic-IAT. A: male participants, B: female participants *Error bars* indicate standard errors mean over participants * $p < 0.05$. Notes: tDCS. Transcranial direct current stimulation; EVC. Extrastriate Visual Cortex; IAT. Implicit association test.

Title: Cathodal transcranial direct current stimulation of the extrastriate visual cortex modulates implicit anti-fat bias in male, but not female, participants

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Abstract

Explicit negative attitudes towards obese individuals are well documented and seem to modulate the activity of perceptual areas, such as the Extrastriate Body Area (EBA) in the lateral occipito-temporal cortex, which is critical for body-shape perception. Nevertheless, it is still unclear whether EBA serves a role in implicit weight-stereotypical bias, thus reflecting stereotypical trait attribution on the basis of perceptual cues. Here, we used an Implicit Association Test (IAT) to investigate whether applying transcranial direct current stimulation (tDCS) over bilateral extrastriate visual cortex reduces pre-existing implicit weight stereotypical associations (i.e. “Bad” with Fat and “Good” with Slim, valence-IAT). Furthermore, an aesthetic-IAT, which focused on body-concepts related to aesthetic dimensions (i.e. “Ugly” and “Beauty”), was developed as a control condition. Anodal, cathodal, or sham tDCS (2 mA, 10min) over the right and left lateral occipito-temporal (extrastriate visual) cortex was administered to 13 female and 12 male participants, before performing the IATs. Results showed that cathodal stimulation over the left extrastriate visual cortex reduced weight-bias for the evaluative dimensions (Bad vs. Good) as compared to sham stimulation over the same hemisphere. Furthermore, the effect was specific for the polarity and hemisphere of stimulation. Importantly, tDCS affected the responses only in male participants, who presented a reliable weight-bias during sham condition, but not in female participants, who did not show reliable weight-bias at sham condition. The present results suggest that negative attitudes towards obese individuals may reflect neural signals from the extrastriate visual cortex.

Keywords: anti-fat bias; Extrastriate visual cortex; tDCS; Implicit Association Test

Introduction

There is mounting research evidence that overweight and obese people experience social disadvantages in a multitude of social settings, such as interpersonal relationships, employment, education and healthcare (Puhl and Brownell, 2001; Schupp and Renner, 2011). Indeed, various explicit measures have revealed that being overweight or obese is usually associated with a range of negative features, such as being unattractive, weak-willed and sexually estranged (Crandall, 1994; Phillisp and Hill, 1998; Todorov and Uleman, 2003; Todorov et al., 2008). Furthermore, those negative attitudes towards obese individuals (anti-fat bias) seems to develop in early childhood and it has been even observed in children as young as 3 years old, gradually increasing after that (Cramer and Steinwert, 1998).

More recently, this anti-fat bias has been detected (Teachman et al., 2003; Ahern and Hetherington, 2006; Schwartz et al., 2006) by applying "implicit" measures, such as the Implicit Association Test (IAT; Greenwald, Nosek and Banaji, 2003), which can provide an index of the automatic association between the face and body of an obese or slim individual and an evaluative dimension (e.g., Good vs. Bad). Interestingly, participants have shown before higher levels of implicit, as compared to self-report measures, of bias, thus suggesting that the IAT can reveal levels of prejudice that may not be otherwise apparent (Wang, Brownell and Wadden, 2004). These implicit negative attitudes toward overweight and/or obese individuals can then trigger a range of discriminative, non-verbal behaviours, for example eye contact and spatial distance. Such immediate negative behaviours may take place in the absence of reflective thinking (Todorov and Uleman, 2003), thus providing a constant source of discrimination elicited by the mere sight of an obese person (Schupp and Renner, 2011).

Human beings naturally rely on fundamental cues, such as race, sex and age, in order to categorize others (Fiske, 1993), however these cues may elicit stereotypes about the groups they represent and thus, yield person-perception processes (Kunda and Thagard, 1996; Macrae et al., 1994). As such, body shape is an important cue to form impressions of other people on the basis of basic perceptual processing. It is still unclear, however, to what extent body-weight negative stereotypes entail only the activity of high-level brain areas involved in evaluative processing or also modulate the activity of brain regions involved in processing visual information conveyed by body shape. In spite of many studies investigating the underlying neural basis of stereotypical attitudes by administering the IAT (e.g., Cattaneo et al., 2011; Crescentini et al., 2014, 2015; Gallate et al., 2011; Gladwin, den Uyl and Wiers, 2012; Chee et al., 2000), only very few studies have so far used neuroimaging and/or neurophysiological techniques to focus on the neural bases of implicit obesity stigma. A seminal fMRI study of Krendl and colleagues (2006) investigated the neural basis of forming either explicit ("Do you like or dislike this person?") and implicit ("Is this a male or female?") judgments of people having well-established stigmatized conditions, such as obesity. The authors of the study proposed the activation of an extensive neural network, including the amygdala, insula, anterior cingulate, and lateral prefrontal cortex, that is involved in the processing of highly negative social stigmas. These brain areas have been shown before to be also involved in responding to aversive stimuli, as well as modulating inhibition and cognitive control. More recently, Azevedo et al. (2014) reported decreased neural reactivity as a result of observing obese people's pain in areas associated with the representation of sensory and affective-motivational aspects of pain (i.e. bilateral insula, somatosensory cortices and thalamus), revealing diminished resonance with obese people's pain.

In a similar vein, Schupp and Renner (2011) investigated the neural bases of implicit anti-fat bias by means of event-related potential (ERP) recordings. In this study schematic portrays of underweight, normal weight, and overweight body shapes, as well as pictures of tools, served as the stimuli. During a first passive viewing task, participants were asked to simply observe the stimuli, while in a subsequent distraction condition participants were asked to detect a specific tool. The authors reported that observing overweight in comparison to normal-weight or underweight body shapes elicited a positive potential shift over fronto-central sites and a relative negative potential over occipito-temporal regions in a time window from ~190 to 250 msec. Moreover, there was no modulation reported during later time windows. These findings are in accordance with those showing that an early differential ERP activity may be associated with the emotional processing of pictures, faces and words (Wieser et al., 2010) and suggest that the perception of images of obese individuals can modulate early perceptual processing areas, reflecting the intrinsic significance of stimuli (Schupp and Renner; 2011; Wieser et al., 2010). In line with this view, a recent fMRI study of Quadflieg et al. (2011) investigated whether early perceptual aspects of person construal are sensitive to the stereotype-related status of individuals. The authors found that the presentation of targets that violated stereotypic beliefs (e.g., male hairdressers and female airline pilots) increased neural activity not only in areas dedicated to executive control (i.e., DLPFC), but also in areas related to person perception (i.e., FFA, FBA, EBA). These findings suggest that stereotypic beliefs modulate the activity of extrastriate areas involved in person percept in the brain. However, they do not provide evidence on how modulation of activity in these areas contributes to the formation and reshaping of social biases.

To address this issue, we applied transcranial direct current stimulation (tDCS), a non-invasive brain-stimulation technique that can interfere with cerebral cortex processes by means of a weak electric current passed between two electrodes (anodal and cathodal) on the scalp. This way, decreased (cathodal) or enhanced (anodal) cortical excitability can be induced. We used tDCS to directly manipulate the cortical excitability of the extrastriate visual cortex, including the extrastriate body area (EBA), which has been shown to respond selectively to photorealistic depictions of whole human bodies or body parts, still images of human bodies or body parts extending to 'stick figures' and silhouettes, in preference to human faces, images of objects parts and scenes (Downing et al., 2001; Candidi et al., 2008; Peelen and Downing, 2007; Urgesi et al., 2007a).

In two separated sessions, we applied anodal- (a-), cathodal (c-), or sham-tDCS over the extrastriate visual cortex in the right and left hemispheres of male and female participants with the aim of investigating its role in mediating implicit negative weight stereotypical associations (i.e. "bad" with overweight and "good" with slim) as measured with a weight-related valence-IAT (v-IAT). Furthermore, an ad-hoc IAT, which focused on body-concepts related to aesthetic perception (i.e. "ugly" with overweight and "beautiful" with slim), was developed as a control task (aesthetic-IAT, ae-IAT). In particular, in these weight-related IATs, participants were required to classify the body of obese and thin people as Fat and Slim, respectively. In parallel, they were required to classify a series of adjectives along two evaluative dimensions (Good vs. Bad or Beautiful vs. Ugly). In one (congruent) block, bodies and adjectives were randomly presented, while Slim categorization responses were mapped onto the same response key of Good (or Beautiful) categorization responses, whereas Fat and Bad (or Ugly) shared the same response key. In another (incongruent) block, response mapping was inverted, so that the Fat categorizations were mapped with the Good (or Beautiful) ones and the Thin with the Bad (or Ugly) categorizations. In keeping with previous studies (Teachman et al., 2003; Ahern and Hetherington, 2006; Schwartz et al., 2006), we expected participants to be faster to respond in the first pattern than in the second one, which is taken as evidence of ‘anti-fat bias’.

In line with Greven, Downing, and Ramsey (2016), Greven and Ramsey (2017) and Quadflieg et al. (2015), we expected that neural activity in extrastriate visual cortex (and particularly in EBA) should inform the so-called “core person perception network” (Gobbini and Haxby, 2007; Weiner and Grill-Spector, 2010 and Greven et al., 2016) about bodily appearance, thus selectively modulating the associations between implicit personality judgments with weight-bias. Predictions regarding the direction of the after-effects of c- and a-tDCS on occipito-temporal areas should be cautious, as they appear to be task-dependent and are still controversial (Antal, Nitsche, and Paulus, 2006). However, based on the results of Quadflieg et al. (2011) showing increased activity of EBA for stereotype-incongruent depictions of human bodies, we expected that inhibiting excitability of extrastriate visual cortex with c-tDCS should reduce implicit anti-fat bias, whereas facilitating excitability of extrastriate visual cortex with tDCS should increase it. Furthermore, comparing the effects obtained for the two weight-related IATs may allow us to verify if the role of the extrastriate visual cortex is merely related to the perception of body weight (i.e., with comparable effects of tDCS for the v- and ae-IAT) or reflects higher-level involvement in associating specific evaluative dimensions to body forms (i.e., with selective effects for one IAT). Finally, tDCS effects should be influenced by the interindividual differences in implicit and explicit weight-related stereotypes that are expected between men and women (Lieberman, Tybur and Latner, 2012), with men reporting more negative general attitudes toward obese individuals than women and, consequentially, specific reduction or increase of implicit anti-fat bias after c- or a- tDCS, respectively.

Methods

Participants

A total of 25 students (13 women, range: 20-29 years old; 12 men, range: 20-28 years old) from the University of Udine, Italy, participated in the experiment in return for course credits. Participants were naïve as to the purpose of the study and information about the experimental hypothesis was provided only during the debrief period, after all the experimental tests were completed. All subjects, but one male and one female, were right-handed as identified by means of a Standard Handedness Inventory (Briggs and Nebes, 1975). They were all native Italian speakers of Caucasian race and they all reported heterosexual orientation. Finally, all participants reported normal or corrected to normal vision, they were in good health, free of psychotropic or any other medication, with no past history of psychiatric or neurological disease and with no contraindication to tDCS (Poreisz et al., 2007). At the end of the experiment, participants filled two questionnaires: 1) the Sociocultural Attitudes Toward Appearance Questionnaire-3 (SATAQ-3; 4 scales; Stefanile et al., 2011) to measure multiple aspects of societal influence, such as the degree of mass media internalization of the models; 2) the Fat Phobia scale (short version from Bacon et al., 2001) in order to measure fat phobic attitudes. In particular, The Fat Phobia Scale – short form (Bacon et al., 2001) assesses explicit negative attitudes and stereotyped perceptions of obese people. This scale consists of 14 pairs of adjectives that are sometimes used to describe obese individuals. For each pair, participants have to indicate, using a 5-point scale, the adjective that best describes their feelings and believes (e.g. 1 = Industrious/5 = Lazy). Higher scores reflect greater fat phobia. Furthermore, we estimated participants' BMI from self-report measures of weight (Kg) and height (cm). The participants' demographics and self-report questionnaire scores as a function of gender are reported in Table 1. Participants gave their written informed consent and all experimental procedures were previously approved by the ethics committee of the Scientific Institute (IRCCS) 'E. Medea' and were in accordance with the ethical standards of the Declaration of Helsinki (1964).

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Materials and Methods

Body Stimuli

All participants were shown a series of 6 virtual human models (3 females / 3 males) previously selected from a database of adult body stimuli created by means of Poser Pro 2010 (e-frontier, Santa Cruz, CA) (for details see Cazzato et al., 2012). Virtual models rather than “real” persons were used in order to limit confounds related to differences in attractiveness, clothing, attire, and familiarity (Schupp and Renner, 2011). The coloured virtual models were rendered in two different static daily poses (e.g., standing). The body weight was gradually increased or decreased in order to create two body size extremes for each model (fat/slim). All pictures were taken with the models standing in frontal-view, against a grey background and wearing identical black clothing (underwear). Following that, photorealistic textures were applied and the images were rendered with global illumination. Finally, in order to avoid the influence of any facial features, the pictures were imported into Adobe Photoshop 7.0 (Adobe System Inc. CA; http://www.adobe.com) and a circle region around the face was scrambled.

IAT words

A pilot study was run to appropriately select words stimuli for the valence (good and bad) and aesthetic (beautiful and ugly) categories, which were used respectively in the v-IAT and ae-IAT**.** The entire corpus of evaluative- and aesthetics-related adjectives was selected among a larger sample of words contained in the COLFIS database (CoLFIS database: Corpus and Frequency Lexicon of Written Italian, Bambini and Trevisan, 2012). An independent group of 25 Italian subjects (9 males and 16 females; range: 18-36 years old), who did not take part in the tDCS experiment, rated each word (n=94) on a series of 7-point Likert scale by judging: 1) familiarity (subjective report about how frequently a word occurs in the life of a person); 2) imageability (ease and speed of a word in evoking a mental image or a sensory experience); 3) concreteness (reference to objects, living things, actions and materials that can be experienced through the senses); 4) valence (ability of a word to elicit in the speaker and listener positive or negative feelings) and 5) strength of association of each adjective with aesthetic and valence dimensions. Table 2 reports the mean values for each of the above-mentioned dimensions for the four categories of stimulus words. A total of final forty-eight words (12 for each category) were selected as stimuli (see Table 3). A series of one-way ANOVAs on each dimension indicated that the categories were matched for familiarity [$F(3,44) = 2.130$, $p = 0.110$, $\eta p^2 = 0.127$], imageability [$F(3,44) = 2.540$, $p < 0.069$, $\eta p^2 = 0.148$], length of letters [$F(3,44) = 1.321$, $p = 0.280$, $\eta p^2 = 0.083$] and frequency of word use in Italian language (COLFIS database) [$F(3,44) = 1.145$, $p = 0.341$, $\eta p^2 = 0.072$], but not for concreteness [$F(3,44) = 13.954$, $p < 0.001$, $\eta p^2 = 0.488$]. Newman-Keuls post hoc tests for the concreteness measure showed that the words used in the aesthetic category (Beautiful and Ugly) were judged more concrete than the other two categories of words (Valence: Bad and Good) (all $p < 0.001$). Importantly, the analysis on valence ratings revealed a main effect of category [$F(3,44) = 326.896$, $p < 0.001$, $\eta p^2 = 0.957$], with Beautiful and Good words having more positive valence than the other two types of words (all $p < 0.001$). Finally, the analysis on the strength of association (difference between the association of each word with the aesthetic and valence dimensions) confirmed that Beautiful and Ugly words were more associated with the aesthetic then valence dimension and that Good and Bad words were more associated with the valence than the aesthetic dimension [$F(3,44) = 42.393$, $p < 0.001$, $\eta p^2 = 0.743$; all $p< 0.001$]. Thus, the pilot experiment confirmed the validity of our measures of aesthetic and valence representations.

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Experimental Procedure

During the experiment, participants were seated in a dimly light room at a distance of approximately 57 cm away from a LCD monitor (19 inches, resolution of 1024*768 pixels, refresh frequency at 60 Hz)**.** The experiment was designed and controlled with E-Prime software (version 2.0 Professional, Psychology Software Tools, Inc., Pittsburgh, PA). At the beginning participants had to complete their demographic details, followed by brief written instructions about the task and, then, by the v-IAT. Participants were instructed to respond as fast and accurate as possible immediately after the onset of the stimuli (i.e., single words or images presented one at a time at the centre of the screen), by pressing a left (E) or a right (I) key on the computer keyboard with the index finger of their left and right hand, respectively. Each IAT lasted approximately 8 minutes and was administered in seven blocks, each consisting of both congruent and incongruent condition blocks (blocks 3, 4, 6, and 7) and familiarization blocks (blocks 1, 2, and 5) (Greenwald, 2003; Cattaneo et al., 2011; Crescentini et al., 2014). Before the first running of each IAT, participants were shown a list with all the words belonging to the two relevant categories and they were asked to carefully study all the stimuli.

In the first block of v-IAT, 12 images of Fat and 12 images of Slim people were presented and had to be classified as being either Fat (left key) or Slim (right key). Each of the 12 images of the two categories was presented only once for a total of 24 trials. The second block also consisted of 24 trials, in which Bad-related (requiring a left-key response) and Good-related (requiring a right-key response) words were presented. In the third block (24 practice trials) and in the fourth block (48 test trials), both Fat and Slim bodies and Good and Bad words were randomly presented and participants were instructed to press the left key for Bad-related words and images of Fat people, and the right key for Good-related words and images of Slim people (congruent-stereotype condition). In the fifth block (24 trials), response key assignments were reversed in relation to the categorization involving images of fat people (right key) and images of slim people (left key). Finally, in the sixth block (24 practice trials) and in the seventh block (48 test trials), both Fat and Slim bodies and Good and Bad words were randomly presented and participants were required to press the left key for images of Fat people and Good words and the right key for images of Slim people and Bad words (incongruent-stereotype condition) (see Table 3). Typically, participants are faster and more accurate in the congruent- than in the incongruent-stereotype blocks, thus demonstrating an automatic association between Fat and Bad categories and Slim and Good categories (Greenwald, Banaji and Nosek, 2003).

With regards to the control ae-IAT, the procedure was exactly the same as the v-IAT, with the exception that aesthetics-related words were presented and participants were instructed to classify the words as being related to Beautiful or Ugly categories (see Table 3). The 12 images of fat and slim people presented during the v-IAT were also used in the ae-IAT. Stimuli within each block were presented in a random order. Each stimulus (word/image) persisted on the computer screen until the participant gave a correct response. If participants made an error, then a red “X” appeared below the word stimulus in order to prompt them to correct the mistake by pressing the correct key. Following the response, the next stimulus appeared after 500 msec, during which only the category labels were visible on the screen. In two separate days (one per each hemisphere), the two IATs were presented to each participant in three blocks, one for each of the stimulation type (sham, a- and c-tDCS). Each block lasted for about 20 min (tDCS stimulation + task duration). Moreover, half of the participants performed first the v-IAT and second the ae-IAT; the opposite order was used for the other half. Finally, after the tDCS experiment, participants were required to provide information about their weight and height (for calculating BMI) and to complete the SATAQ-3 and Fat Phobia Questionnaires.

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tDCS

Anodal, cathodal or sham-tDCS (2 mA) was delivered by means of a battery-driven, constant-current stimulator (BrainStim, EMS, Bologna, Italy) through a pair of saline-soaked sponge electrodes (5 × 5 cm, 25 m^2).

The electrodes were first firmly attached by elastic bands and saline solution was applied under the electrodes in order to reduce contact impedance before the montage. To comply with current safety regulations (Poreisz et al., 2007), a constant current of 2 mA intensity was applied. Specifically, the stimulating current was ramped up during a 10-sec fade-in phase, then held constant at 2 mA for 10 min, and then ramped down during a 10-sec fade-out phase. We chose this specific duration of the tDCS stimulation on the basis of previously reported experimental protocols, which have described effects on cortical excitability, sufficiently enduring to cover the duration of the experimental task (Nitsche and Paulus, 2001; Mancini et al., 2012). The experimental task was initiated exactly in the last 2 min of tDCS. In each daily session, the participants received a-, c-, and s-tDCS on the same hemisphere in three separate blocks. The order of the hemisphere daily sessions and of the stimulation-condition blocks was counterbalanced across subjects. An interval of 3-5 days was allowed between the two daily sessions and of at least 90 min between the three stimulation-condition blocks in order to avoid carryover effects and to guarantee a sufficient washout of the effects of the previous session (e.g., Mancini et al., 2012; Bolognini, et al., 2010; 2011). During the 90 minutes of break, participants were free to leave the laboratory and take some rest. During the three different experimental blocks, the location of the active electrode was identified by means of the 10–20 system for EEG electrode placement. In keeping with previous studies targeting the lateral occipito-temporal cortex with tDCS (Mancini et al., 2012), the active electrode was placed between O2 and PO8 to stimulate the extrastriate visual cortex, including visual body-specific regions (Mancini et al., 2012; Downing et al., 2001). The reference electrode was always fixed on the vertex (Cz). Moreover, as in previous studies, for the sham condition, the electrodes were placed over the target sites (see Fig. 1), with the same parameters of a- and c-tDCS, but the stimulator was turned off after 30 sec (Nitsche and Paulus, 2000; Mancini et al., 2012). This ensured that participants could initially feel the itching sensation at the beginning of the tDCS protocol, but no effective modulation of cortical excitability could be elicited (Gandiga, Hummel and Cohen, 2006). Finally, in-house software switched the tDCS on and off without intervention from the participants or experimenters, allowing for successful blinding.

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Data Handling

Statistical analyses were performed on the mean D-scores, which measure the IAT effects by combining both the accuracy and speed aspects of responses and were computed following the improved algorithm procedure described by Greenwald et al. (2003) and Cattaneo et al. (2011). In particular, we first checked that there were no trials with latencies greater than 10,000 msecs and no participants responded faster than 300 msecs in more than 10% of all the experimental trials. Then, for computing the mean reaction times (RTs), RTs of error trials were removed and replaced with the mean RTs of correct trials in the corresponding block plus an addition of 600 msec. To compute D-scores, the mean RTs of block 3 were subtracted from the mean RTs of block 6 and the difference was divided by the pooled SD of all trials in blocks 3 and 6; similarly, the mean RTs of block 4 were subtracted from the mean RTs of block 7 and the difference was divided by the pooled SD of all trials in blocks 4 and 7. Finally, the two quotients obtained in the previous two steps were averaged (Cattaneo et al., 2011). For the sake of clarity, error rates and RTs of correct responses are reported in Table 4, respectively for each IAT.

First, we tested whether male and female participants presented with significant weight bias in the two IATs at the baseline (sham) condition by comparing the corresponding mean D-scores to zero (where zero refers to the absence of any response bias). Then, to test the effects of tDCS on the implicit association of weight to good/bad attributes and to control beautiful/ugly attributes, the D-score data were entered into two separated mixed-model Analyses of Variance (ANOVAs), one for each IAT, with gender group (male, female participants) as between-subjects factor and tDCS stimulation (anodal, cathodal, sham) and Hemisphere (left, right) as within-subject variables. Significant three-way interactions were followed up by separate 2-way ANOVAs in each gender group, while the source of significant two-way interactions was analysed using the Newman-Keuls post-hoc test.

Finally, we calculated, for each condition, a measure of the change of v-IAT D-scores as the difference between the individual values after c- and a-tDCS and the corresponding values in the sham-tDCS condition [active-tDCS - sham-tDCS]. The change indexes were correlated, using Pearson correlations, with BMI and individual scores at the Fat Phobia Scale and SATAQ questionnaire.

All statistical analyses were performed with STATISTICA 8.0 (StatSoft Inc, Tulsa, Oklahoma). Effect sizes were estimated using the partial eta square variable (ηp^2). All data are reported as Mean (M) and Standard Error of the Mean (s.e.m.). A significance threshold of $p < 0.05$ was set for all effects.

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Results

Valence-IAT

One sample t-tests comparing the mean D-scores to zero showed that male participants showed a significant stereotypical anti-fat bias in both sham-tDCS conditions, indicating that they were more prone to associate fat people to the bad-related category and slim people to the good-related category than vice versa [$t(11) = 3.56$, $p = 0.004$ for right sham-tDCS and $t(11) = 5.04$, $p < 0.001$ for left sham-tDCS]. Conversely, the analysis of the female participants' mean D-scores revealed absence of the anti-fat bias in both sham-tDCS conditions, namely for right [$t(12) = 1.15$, $p = 0.271$] and for left sham-tDCS [$t(12) = 1.61$, $p = 0.134$].

The 3-way ANOVA on the v-IAT revealed a significant 3-way interaction of hemisphere × tDCS stimulations × gender [$F_{(2,46)} = 3.356$; $p = 0.044$; $\eta_p^2 = 0.127$]. The follow-up 2×3 ANOVA on the mean D-scores for male participants revealed a significant *2*-way interaction of hemisphere × tDCS stimulations [$F_{(2,22)} = 7.522$; $p = 0.003$; $\eta_p^2 = 0.406$], but no main effects of hemisphere [$F = 0.794$, $p = 0.392$] or stimulation [$F = 0.924$, $p = 0.412$]. Newman-Keuls post-hoc comparisons showed that c-tDCS over left extrastriate visual cortex reduced the weight-bias for the v-IAT, as compared to sham [0.13 ± 0.7 vs. 0.44 ± 0.9, $p = 0.007$]. The effect was specific for the polarity and hemisphere of stimulation, since the weight-bias after c-tDCS over the left extrastriate visual cortex was significantly lower than that after c-tDCS over the right extrastriate visual cortex [0.13 ± 0.7 vs. 0.37 ± 0.06; $p = 0.035$; see Fig. 2A]. Crucially, the difference between the two sham conditions in the right and left hemisphere stimulation sessions was not statistically significant [0.26 ± 0.07 vs. 0.44 ± 0.09; n.s.].

-------------------------------- Please insert Fig. 2 around here --------------------------------

The 2×3 ANOVA on the mean D-scores of female participants revealed non-significant main effects of hemisphere and stimulation and non-significant interaction between the two factors [all *Fs* < 1.367, all *ps* > 0.274] (See Fig. 2B).

Aesthetic-IAT

At baseline, male participants showed a significant stereotypical anti-fat bias in both sham-tDCS conditions, indicating that they were more prone to associate fat people to the ugly-related category and slim people to the beautiful-related category than vice versa [$t(11) = 0.29$, $p = 0.007$ for right sham-tDCS and $t(11) = 0.40$, $p < 0.001$ for left sham-tDCS]. The analysis of the female participants' mean D-scores revealed a significant anti-fat bias in both sham-tDCS conditions, namely for right [$t(12) = 0.3$, $p = 0.015$] and for left sham-tDCS [$t(12) = 0.34$, $p = 0.010$]. Thus, the aesthetic anti-fat bias was apparent in both gender groups.

However, the 3-way ANOVA on the ae-IAT D-scores (Fig. 3) revealed non-significant main effects or interaction [all $F < 1$; $\eta_p^2 < 0.031$]. In particular, the non-significant 3-way interaction between gender group, hemisphere, and stimulation [$F_{(2,46)} = 0.199$; $p = 0.821$; $\eta_p^2 = 0.009$] suggests that the gender- and hemisphere- specific modulation of the weight-bias in the valence dimension was not reflect in the aesthetic dimension.

-------------------------------- Please insert Fig. 3 around here --------------------------------

Self-reported questionnaires

As shown in Table 1, independent sample t-tests indicated that male and female participants were matched for both age and BMI. The analysis of the SATAQ-3 data revealed that, compared to women, men had higher scores on the internalization-athlete

SATAQ-3 subscale, thus indicating that they might have a stronger internalization of media influences related to the achievement of an athletic physique (Internalization-Athlete); conversely, the two gender groups did not differ on the thin-ideal internalization score (Internalization-General), the perceived feelings of pressure to conform to the Western ideals exhibited by the media (Pressures) and the recognition of the social importance of the media's messages about Western beauty ideals information (Information). Furthermore, no differences were found between men and women in the explicit phobic attitude towards fat people. Finally, no significant correlations were found between the tDCS change indexes and the BMI, Fat Phobia, and SATAQ subscales for both male and female participants ($-0.612 <$ all rs < 0.433).

Discussion

This study applied tDCS to examine whether non-invasive brain stimulation can modulate anti-fat bias, and we demonstrated that stimulation over the left, but not right, extrastriate visual cortex, where EBA has been previously located (Sadeh et al., 2011; Taylor et al., 2010), decreased negative attitude towards fat people. Importantly, we also developed a control ae-IAT, which focused on body-concepts related to aesthetic representations (i.e. "ugly" and "beauty") and we found that inhibiting neural excitability in the left occipital cortex by applying c-tDCS diminished the anti-fat bias only for the v-IAT but not for the ae-IAT. Conversely, enhancing cortical excitability through a-tDCS did not exert any effects in either hemisphere. Interestingly, the effects of tDCS for the v-IAT were found only in male participants, who displayed a significant anti-fat bias, but not in female participants, who did not show a reliable anti-fat bias. To the best of our knowledge this is the first study showing a causative role of the lateral occipito-temporal cortex in the anti-fat bias.

Consistently with previous research (Puhl, Luedicke, and Heuer, 2011; Musher-Eizenman, and Carels, 2009), dominant implicit representations of obese individuals as dishonest, villain and immoral were evident at sham condition. The weight v-IAT effect, however, was only significant in male but not in female participants, suggesting a lack of implicit anti-fat bias in women even if no differences were found between men and women in their explicit fat phobic attitudes. Nevertheless, the absence of a significant implicit weight bias in female participants allowed for an indirect control for general effects of tDCS on the IAT performance in the absence of any reliable weight bias. Importantly, this result seems to be in agreement with previous experimental evidence suggesting a strong prevalence of negative attitudes towards overweight individuals and, in general, of social stigma in men as compared to women (Lewis, Cash and Bubb-Lewis, 1997). For example, some earlier studies have shown that African-American individuals, particularly women, may be less likely than Caucasians to hold negative attitudes about obese people (Hebl and Heatherton, 1998; Perez-Lopez, Lewis and Cash, 2001). Furthermore, Wang et al. (2007) found that men held more stigmatizing attitudes towards mental illness than women. Most importantly, the reason behind sex differences in obesity stigma may reside in the different motivations in the two genders: women usually report significantly greater fear of becoming fat than men do; in contrast, men are significantly more likely to attribute obesity to a lack of willpower and to report greater dislike of obese individuals as compared to women. This is true even after controlling for BMI (Lieberman, Tybur, and Latner, 2012). Hence, future studies should take into consideration specific subtypes of anti-obesity attitudes that may show systematic sex differences, as this is particularly important for future intervention implications (Kelly and Jorm, 2007).

Importantly, after c-tDCS over left extrastriate visual cortex, the men's negative bias for stereotype-congruent stimuli was reduced, revealing that the anti-fat bias requires the contribution of this brain area. That the inhibition of left extrastriate cortex induced a disruption of the congruency-stereotype association is in line with previous evidence about implicit processing of emotional faces, showing that inhibiting with c-tDCS the activity in the left occipital cortex suppressed the facilitation of responses to emotional faces that was induced, after sham tDCS, by presenting congruent vs. incongruent/neutral masked faces (Cecere, Bertini and Ladavas, 2013). This documents the crucial role of the left occipital cortex in mediating high-order implicit visual processes, such as the emotion congruency effects (Cecere, Bertini and Ladavas, 2013).

It has been previously shown that the extrastriate visual cortex and the functional localized EBA is causatively involved in mapping morphological features of human bodies (Downing et al., 2001; Candidi et al., 2008; Urgesi et al., 2007a). This process can prove critical for maintaining constant the identity of others, even when body configurations change drastically during action sequences. Thus, the role of EBA may be fundamental for the identification of actors, particularly when facial cues are unavailable or ambiguous. Indeed, several studies have shown that EBA is sensitive to subtle variations of human body size and shape (Aleong and Paus, 2010) in healthy individuals and its neuro-functional alteration is associated with body image disturbance, such us body size overestimation and negative evaluation of one's own body, in patients with Eating Disorders (ED) (Uher et al., 2005). The present study shows that neural activity in the extrastriate visual cortex, and possibly EBA, may play a role in contributing to implicit weight-stereotypical bias, reflecting top-down modulation due, for example, to increased attention towards fat as compared to thin bodies. Hence, our results extended previous knowledge (e.g., Quadflieg et al., 2011) on the role of perceptual processing areas in social biases by showing that artificially modulating the neural excitability of extrastriate visual areas implicated in the evaluation of body shape (Urgesi et al., 2007b; Downing et al., 2001) can change prejudice towards fat people.

Conversely, a-tDCS of the extrastriate visual cortex did not modulate the anti-fat bias. Anodal-tDCS has been shown to enhance perceptual (Falcone et al., 2007) and motor (Nitsche et al., 2003) learning, social ability (Santiesteban et al., 2012), and visual analgesia (Mancini et al., 2012). However, studies using tDCS in animal models (Bindman et al., 1964; Creutzfeldt, Fromm and Kapp, 1962) have shown that the effect of cathodal stimulation is stronger than the effect of anodal stimulation if identical stimulation parameters are used. This is in line with the general observation of asymmetric neuroplastic effects in the central nervous system, with excitability reductions being easier to elicit than excitability increases, as shown in animals in vivo (Froc et al., 2000; see Antal et al. 2006 for a review on tDCS effects on visual cortex). Part of the explanation of this asymmetry may reside in the fact that in some experiments the visual system is probably already optimally tuned in healthy subjects and, thus, an excitatory enhancement induced by a-tDCS cannot further improve the perception of visual stimuli (Antal et al., 2006). Overall, these findings support the notion that additional factors, such as the orientation of the electric field (e.g., Nitsche and Paulus,

2000) and the background level of activity in the system when tDCS is applied, might have affected our results. Hence, some features of the task-related activation may interact with the physiological state of the cortex and polarity of tDCS stimulation (Vallar and Bolognini, 2011; Antal and Paulus, 2008; Antal et al., 2004). Further experimental manipulations are deemed as necessary in order to further investigate the potential roles of these actors with respect to the absence of a-tDCS effects over occipital brain areas.

A further result of the present study is that, despite differences between the two gender groups, no relation was observed between the changes of weight bias after c-tDCS and the individual level of explicit phobic attitude and internalization of Western ideals and BMI. This might be due to the fact that the range of observers' BMI and self-report measures within our female and male samples was not large enough to disclose any relevant effects of interindividual differences. This finding, however, is in keeping with a study of Teachman and Brownell (2001) and Teachman et al. (2003), who found no evidence of statistically significant relation between the Fat Phobia Scale and implicit bias as detected with a bad/good weight-IAT that was similar to our task. Such dissociation between implicit and explicit measures of anti-fat bias might result from considering social undesirable the labelling of obese individuals as 'bad' (Teachman and Brownell, 2001).

Limitations

There are a few limitations to consider when interpreting the current findings. First of all, we need to consider that the spatial resolution of tDCS, due to using large sponge pads positioned on the skull, can be relatively diffuse. Considering that, it has been previously reported that brain stimulation by means of tDCS protocols is unlikely to be constrained to the cortex underneath the electrodes (Datta et al., 2009; Bikson and Rahman, 2013; Bestmann, de Berker and Bonaiuto, 2015). As such, we could speculate that inhibition of extrastriate visual cortex might have affected nodes of a broader network involved in person perception and person knowledge. Indeed, it has been previously reported that the frontal cortex, anterior temporal lobes and the limbic system are key areas implicated in the forming of social prejudice. More specifically, the amygdala has been found to be critically involved in cognitive and affective learning, including implicit attitudes (Amodio and Devine, 2006; Dolan et al., 2000; Phelps, Cannistraci, and Cunningham, 2003; Stanley, Phelps, and Banaji, 2008). Furthermore, recent experimental evidence has proposed a critical involvement of the anterior temporal lobes in expressing prejudice by means of conceptual processing (Snyder, Bossomaier, and Mitchell, 2004; Gallate et al., 2011). Finally, a study of Cattaneo and colleagues (2011) demonstrated the causal role of the prefrontal cortex in controlling gender stereotypical beliefs in men. Interestingly, they found that non-invasive brain stimulation delivered at stimulus presentation over the prefrontal cortices led to an increased gender-stereotypical bias for the D-scores of male participants, as compared to a control condition. It therefore remains to be determined how specific the current results are to the stimulation site and, for example, whether interfering with the activity of the extrastriate visual cortex might have in turn interfered with key areas important for the control of automatic (negative) associations, such as the prefrontal cortices.

In a similar vein, we cannot rule out that tDCS may have affected top-down control mechanisms, such as the ability to regulate bias (Conrey et al., 2005) and task-switching abilities (Klauer et al., 2010), that are involved in performing an IAT. Although the gender- and IAT-selectivity of the effects of c-tDCS over left extrastriate visual cortex would speak against general effects on IAT categorization performance, one may speculate that c-tDCS might have affected cognitive control abilities particularly in those individuals (i.e., men) who show higher anti-fat bias and, thus, need more cognitive control to moderate it.

Although order of testing was counterbalanced across participants, one potential limitation could rely on the repetition of the same IAT task and of different tDCS conditions (anodal, cathodal, sham) within the same day/week. Indeed, it has been shown that the magnitude of the effect tends to decline with repeated administrations (Nosek, Greenwald and Banaji, 2007). However, the absence of any repetition effects for the control ae-IAT rules out this possibility.

In a similar vein, the possible mediating role of perceived attractiveness of the body stimuli used during both IATs needs to be considered, Indeed, some researchers have claimed that anti-fat prejudice may stem from the perception of overweight individuals as unattractive or aesthetically displeasing (e.g., Morrison and O'Connor, 1999). However, we found gender differences in the v-IAT even during sham stimulation, but both male and female participants showed reliable implicit weight-bias in the association of fat or slim bodies to the beautiful-ugly dimension in the ae-IAT. Furthermore, tDCS affected men's v-IAT, but no specific tDCS modulation was found for the ae-IAT, suggesting that valence and aesthetic evaluations may be two independent judgement categories during person perception and might be underpinned by different neural circuitry.

Furthermore, during the IAT procedure, participants are explicitly required to classify stimuli according to their body weight. Thus, it is unclear whether body-related perceptual areas are similarly involved when anti-fat bias is prompted by the mere sight of an obese body independently from explicit focus on the weight dimension (Moors and De Houwer, 2006; Schupp and Renner, 2011; see also Quadflieg et al., 2011). Finally, it cannot be determined to what extent the selective decrease in the anti-fat bias after EBA c-tDCS observed in this study can be generalised to other specific subtypes of anti-obesity attitudes and/or social stigma in general. Further studies are required to systematically examine the effects of tDCS on various negative attitudes against stigmatized social groups.

Conclusions

Overall, the present study may contribute to the growing social neuroscience literature on the neural underpinnings of person perception, thus extending previously reported work on explicit and implicit weight stigma as a function of first impression formation (e.g. facial attractiveness, trustworthiness, and competence). Previous neuroimaging studies (e.g., Quadflieg et al., 2011) have shown that early perceptual aspects of person construal are sensitive to the stereotype-related status of individuals. Here, we provided causative evidence that activity in body-selective occipito-temporal areas actively contributes to the formation and expression of implicit stigma based on body size. This pairing of functional responses between distinct brain circuits may indicate that person-perception and person-knowledge neural networks are not entirely encapsulated from other neural brain systems. Recently, Downing and Peelen (2011) in a seminal study have found that the primary function of EBA is grounded on visually analysing the bodies of conspecifics, but also that during this process EBA may also exchange signals with other brain circuits. The present findings, as well those previously reported in the literature (Greven, Downing and Ramsey, 2016; Ewbank et al., 2011; Quadflieg et al., 2011; Zimmermann et al., 2013) can for the first time provide empirical support for this and enhance the belief that interactions between specific neural systems may upregulate or downregulate neural responses in the body-selective cortex.

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Figures Legends:

Fig. 1: Schematic representation of tDCS electrodes montage over left and right Extrastriate visual cortex.

Fig. 2: Effects of cathodal (c-tDCS), anodal (a-tDCS) and sham-tDCS (s-tDCS) on D-scores as a function of gender (men, women) and t-DCS hemisphere (right EVC, left EVC) for the valence-IAT. A: male participants, B: female participants. *Error bars* indicate standard errors mean over participants * $p < 0.05$. Notes: tDCS. Transcranial direct current stimulation; EVC. Extrastriate Visual Cortex; IAT. Implicit association test.

Fig. 3: Effects of cathodal (c-tDCS), anodal (a-tDCS) and sham-tDCS (s-tDCS) on D-scores as a function of gender (men, women) and t-DCS hemisphere (right EVC, left EVC) for the aesthetic-IAT. *Error bars* indicate standard errors mean over participants * $p < 0.05$. Notes: tDCS. Transcranial direct current stimulation; EVC. Extrastriate Visual Cortex; IAT. Implicit association test.

Figure 1
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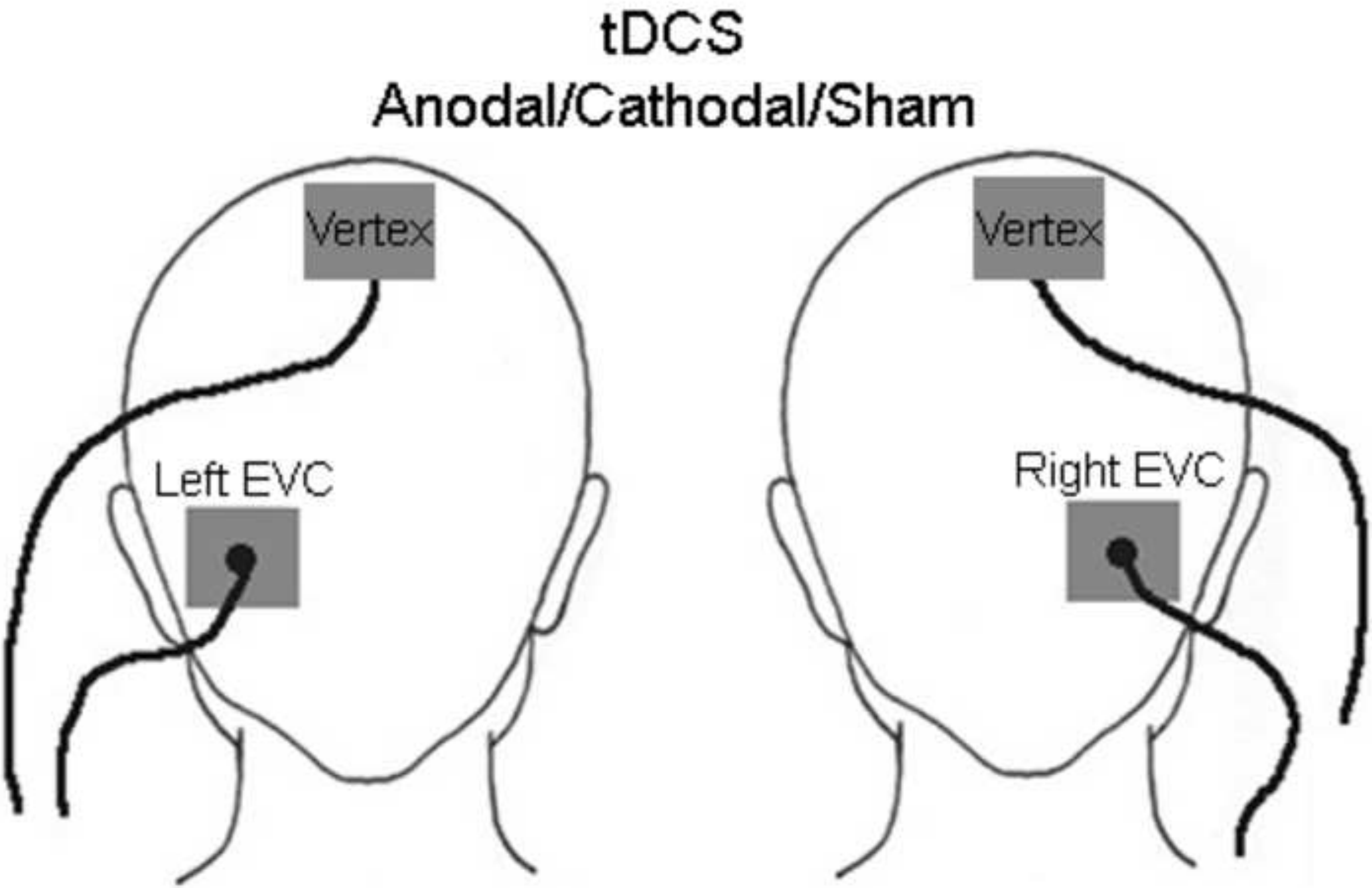

Figure 2

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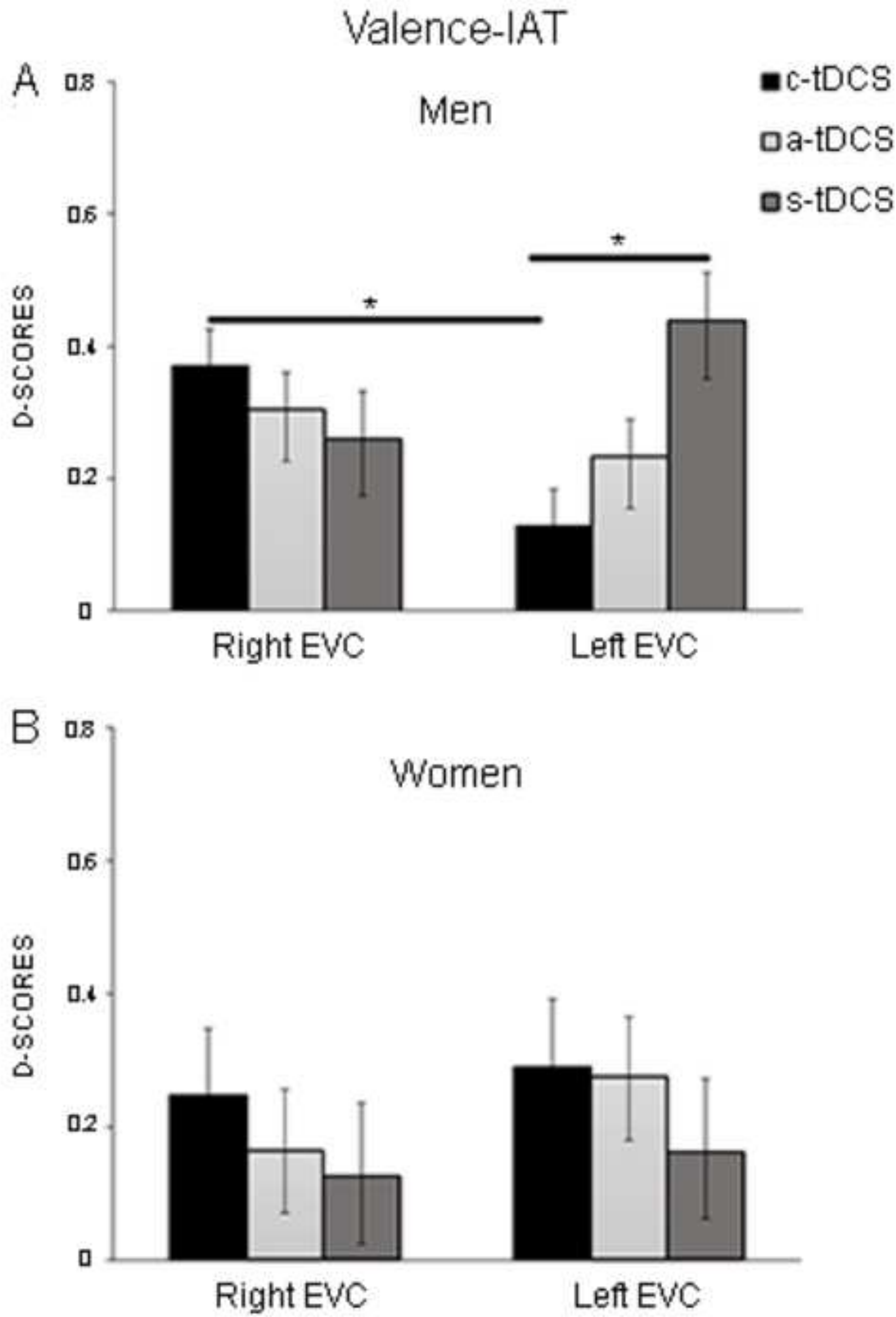

Figure 3
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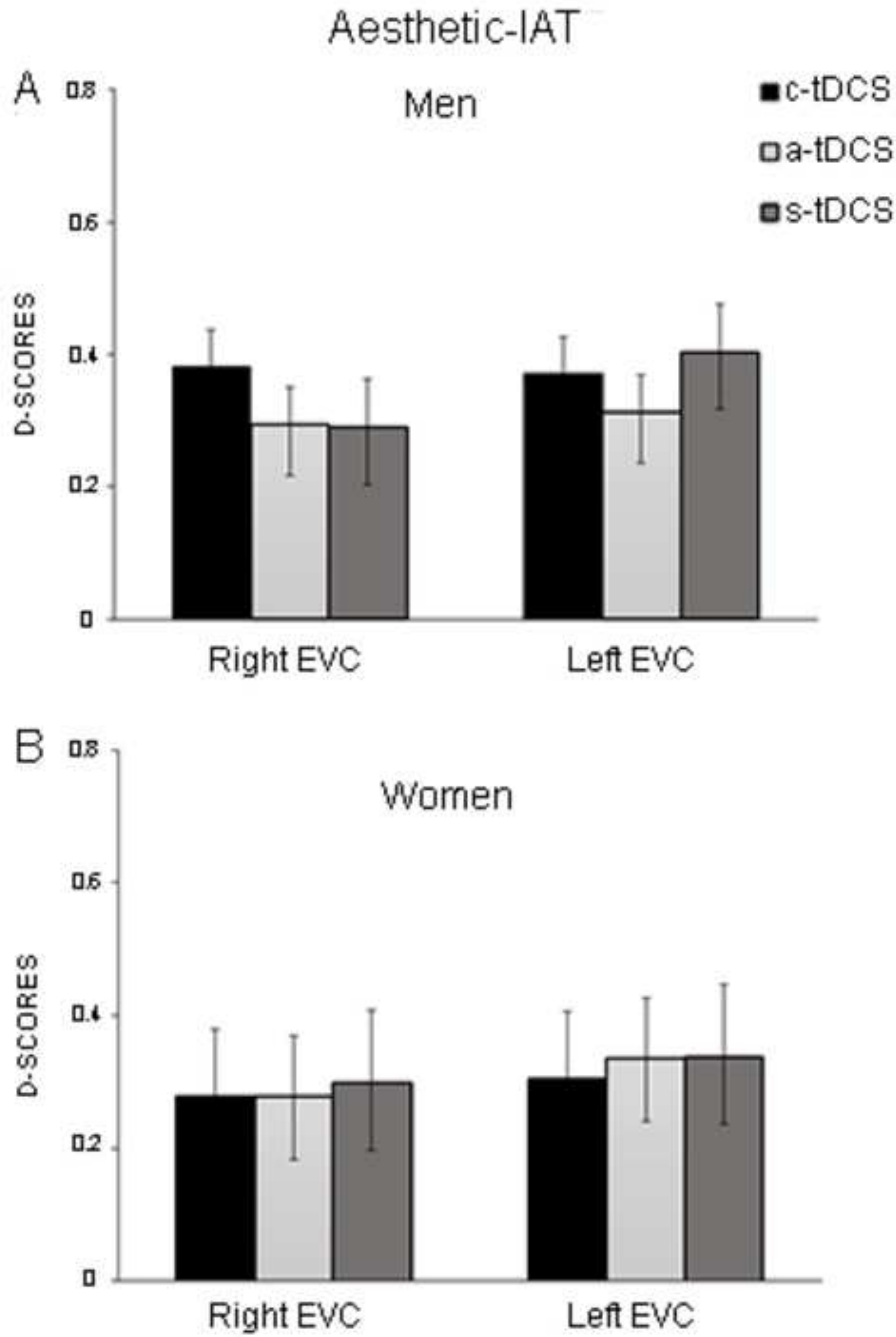

Table

Table 1: Mean and Standard Error of Mean (S.E.M. in brackets) of demographic variables and self-report questionnaire scores for female and male participants.

	Women (n = 13)	Men (n = 12)	Women vs. Men
Age	22.08 (0.73)	22 (0.6)	$t_{(23)} =$ -0.08; p = 0.937
BMI (Kg/cm^2)	21.89 (0.72)	22.77 (0.48)	$t_{(23)} =$ -1; p = 0.328
SATAQ-3			
Information (max 5)	3.48 (0.19)	3.02 (0.28)	$t_{(23)} =$ 1.37; p = 0.182
Pressures (max 5)	2.53 (0.29)	2.05 (0.2)	$t_{(23)} =$ 1.34; p = 0.195
Internalization-General (max 5)	2.26 (0.26)	2.45 (0.24)	$t_{(23)} =$ 0.03; p = 0.975
Internalization-Athlete (max 5)	2.81 (0.28)	3.65 (0.24)	$t_{(23)} =$ -2.6; p = 0.016
FAT PHOBIA SCALE (max 5)	3.62 (0.09)	3.8 (0.08)	$t_{(23)} =$ -1.42; p = 0.17

Notes: The data of the two gender groups were compared by means of independent sample *t*-test (two-tailed). BMI. Body Mass Index; SATAQ-3. Sociocultural attitudes toward appearance questionnaire.

Table 2: Descriptive statistics of the four categories of stimulus words used in the Valence-IAT (Good vs. Bad) and Aesthetic-IAT (Beautiful vs. Ugly).

Adjective category	Imageability	Familiarity	Valence	Concreteness	COLFIS Frequency	Letters	Association Strength
Good	-0.208 (0.136)	0.261 (0.154)	0.957 (0.6)	-0.355 (0.114)	29.917 (4.771)	7.833 (0.39)	0.204 (0.049)
Bad	-0.295 (0.136)	-0.012 (0.154)	-1.017 (0.6)	-0.534 (0.114)	17.917 (4.771)	8.250 (0.39)	-0.315 (0.049)
Beautiful	0.161 (0.136)	-0.149 (0.154)	0.848 (0.6)	0.312 (0.114)	25.417 (4.771)	8.667 (0.39)	0.294 (0.049)
Ugly	0.067 (0.136)	-0.261 (0.154)	-0.947 (0.6)	0.251 (0.114)	21.917 (4.771)	8.833 (0.39)	-0.294 (0.049)

Notes: Mean values (z-scores) and Standard Error of Mean (S.E.M. in brackets) for each stimulus category are based on judgments given on a seven-point scale (Imageability; Familiarity; Valence; Concreteness; and difference between the association strength with aesthetic and valence category) (7 being very imaginable. very familiar. very concrete. very negative. and high associated with Beautiful. Ugly. Good and Bad. respectively). Mean Frequency values are based on the CoLFIS database and mean Letters values are based on the number of letters of the word stimuli.

Table 3: Word stimuli used in the Valence-IAT and in Aesthetic-IAT and (Italian in parentheses). Two categories of words are presented in each IAT.

Valence-IAT		**Aesthetic-IAT**	
Good	**Bad**	**Beautiful**	**Ugly**
Affable (Affabile)	Evil (Malefico)	Charming (Avvenente)	Repulsive (Repulsivo)
Virtuous (Virtuoso)	Dishonest (Disonesto)	Fascinating (Fascinoso)	Clunky (Sgraziato)
Polite (Garbato)	Wicked (Malvagio)	Inebriating (Inebriante)	Repugnant (Ripugnante)
Lovable (Amabile)	Petty (Meschino)	Hunky (Aitante)	Abominable (Abominevole)
Decent (Perbene)	Villain (Infame)	Harmonious (Armonioso)	Horrid (Orrido)
Cordial (Cordiale)	Immoral (Immorale)	Admirable (Mirabile)	Disgusting (Schifoso)
Exquisite (Squisito)	Jerk (Antipatico)	Seductive (Seducente)	Grotesque (Grottesco)
Kind (Cortese)	Malicious (Maligno)	Enchanting (Incantevole	Unpleasant (Spiacevole)
Friendly (Amichevole)	Assertive (Prepotente)	Attractive (Attraente)	Horrendous (Orrendo)
Fabulous (Favoloso)	Perfidious (Perfido)	Pretty (Grazioso)	Revolting (Sgradevole)
Competent (Competente)	Insensitive (Insensibile)	Pleasant (Gradevole)	Monstrous (Mostruoso)
Honest (Onesto)	Brutal (Brutale)	Cute (Carino)	Horrible (Orribile)

Table 4: Mean Reaction Times, Error Rates and Standard Error of Mean (S.E.M. in brackets) for each tDCS site (right/left EVC), type of tDCS stimulation (Cathodal/Anodal/Sham) and for both participants' gender (Men/Women) respectively for the Valence- and Aesthetic-IAT.

			Men (n=12)						**Women (n=13)**					
			Cathodal		**Anodal**		**Sham**		**Cathodal**		**Anodal**		**Sham**	
			Cong	**Incong**	**Cong**	**Incong**	**Cong**	**Incong**	**Cong**	**Incong**	**Cong**	**Incong**	**Cong**	**Incong**
v-IAT	**RTs (msec)**	**Right EVC**	674.55 (24.27)	756.98 (33.48)	675.71 (17.2)	749.38 (40.35)	692.76 (22.5)	741.91 (28.24)	682.78 (30.17)	757.43 (39.53)	761.03 (62.53)	792.27 (52.41)	755.16 (53.42)	767.87 (40.92)
		Left EVC	694.74 (37.53)	708.39 (27.67)	685.26 (26.7)	764.61 (66.29)	658.66 (21.87)	748.05 (34.38)	728.19 (35.03)	816.96 (50.47)	730.55 (33.52)	791.29 (39.49)	736.13 (39.11)	783.96 (50.06)
	ERs (%)	**Right EVC**	1.85 (0.39)	4.51 (0.46)	1.39 (0.51)	3.59 (0.71	2.08 (0.53)	4.51 (0.57)	1.28 (0.4)	2.03 (0.54)	1.71 (0.42)	2.56 (0.7)	1.5 (0.46)	1.71 (0.36)
		Left EVC	1.74 (0.62)	3.13 (0.54)	1.74 (0.42)	3.7 (1.29)	1.5 (0.53)	3.59 (0.77)	2.03 (0.73)	2.46 (0.5)	1.5 (0.48)	1.82 (0.53)	1.28 (0.48)	2.88 (0.6)
ae-IAT	**RTs (msec)**	**Right EVC**	699.32 (42.99)	769.34 (42.7)	683.44 (24.45)	777.94 (38.98)	673.55 (28.42)	757.12 (42.98)	737.67 (53.96)	813.48 (59.54)	726.8 (42.68)	808.45 (62.36)	735.76 (56.71)	808.6 (50.52)
		Left EVC	661.13 (18.37)	746.36 (34.13)	690.01 (42.58)	775.25 (61.8)	673.55 (28.42)	757.12 (42.98)	746.12 (38.45)	826.68 (38.7)	746.18 (43.87)	824.53 (49.01)	731.26 (45.68)	854.15 (85.44)
	ERs (%)	**Right EVC**	1.5 (0.27)	2.89 (0.53)	2.2 (0.6)	4.51 (1.49)	2.55 (0.66)	3.01 (0.72)	1.39 (0.42)	2.78 (0.47)	1.6 (0.41)	2.67 (0.53)	1.5 (0.64)	2.67 (0.64)
		Left EVC	1.97 (0.5)	3.36 (1.06)	0.93 (0.43)	2.31 (0.79)	1.62 (0.48)	3.24 (0.83)	1.92 (0.49)	2.78 (0.96)	1.28 (0.56)	3.42 (0.66)	2.14 (0.6)	3.42 (0.58)

Notes: The data refer to the Mean Reaction Times (in milliseconds, msec); Error Rates (%). EVC. Extrastriate Visual Cortex; Cong. Congruent trials; Incong. Incongruent trial; v-IAT. valence-IAT; ae-IAT. aesthetic-IAT.